Tomato and Pepper Grafting for High Tunnel Production: Effects on Yield, Compatibility, and Plant Morphology

by

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Abstract

Tomatoes and peppers are the most popular and profitable high tunnel crops. However, year-round intensive cultivation and extensive monocropping can lead to a loss of soil quality and the buildup of soilborne pathogens. Many growers are considering grafting to help address the drawbacks of covered agriculture and improve yields. Although many trials have been conducted that examine the ability of rootstock to increase yield or reduce disease, the effect of scion cultivar has yet to be tested, and few studies have attempted to quantitatively assess scion compatibility. In 2016 and 2017, we evaluated ten hybrid, determinate, red slicing tomato scion cultivars for compatibility with 'Maxifort' rootstock in a three-season high tunnel in Olathe, KS. While all ten varieties were compatible with 'Maxifort', only 'BHN 589', 'Red Deuce', 'Skyway', and 'Tasti Lee' were "highly compatible" and showed significant improvements in marketable yield when grafted. Additionally, when ranked by yield, differences between grafted and nongrafted populations suggest that relative compatibility may be inconsistent between varieties. However, a significant inverse relationship between the yield of the nongrafted plants and the percent yield benefit from grafting indicates that the effect of a rootstock like 'Maxifort' may not be synergistic, with higher performing nongrafted scion varieties benefitting less from grafting than lower performing varieties. 'Red Deuce' and 'BHN 589' are productive, and highly compatible grafted varieties with potential for commercial high tunnel production. 'Primo Red' benefitted the least from grafting but was the highest performing nongrafted variety (outperforming four of the grafted combinations).

Compared to tomatoes, published reports on grafted peppers have been limited and it is unclear whether they provide any advantage in the absence of soilborne disease or environmental stress. Additionally, the use of rootstocks from other solanaceous species outside the *Capsicum* genus for pepper grafting has not been well explored, though the pool of available rootstock options for peppers would be substantially increased if such graft unions proved to be compatible. The goals of a second project were to identify the utility of grafted pepper *(C. annum)* plants for commercial high-tunnel production and to explore the potential for graft compatibility between the *Capsicum* and *Solanum* genera. We grafted 'Karisma' bell peppers onto two *Solanum* cultivars ('Maxifort' and 'Sweetie') and three pepper rootstocks ('Scarface', 'Meeting', and 'Yaocali'). Five trials were conducted in 2016-2017 and utilized a randomized complete block design in all locations. Plants grafted onto Solanum rootstocks displayed symptoms of delayed incompatibility, including significant (78%-89%) reductions in yield (by weight), 59%-93% less plant growth, and 58% less marketability, as well as malformations at the graft union and higher in-field mortality rates. These symptoms were likely due to differences in mature stem anatomy. Plants grafted to 'Scarface' produced 32% greater marketable yield, 15%-18% larger fruit, and 9-12% higher marketability than nongrafted 'Karisma'. The results for 'Yaocali' were similar to 'Scarface', though less conclusive. While 'Yaocali' and 'Scarface' rootstocks may be useful for improving yield in low-stress environments, the use of 'Meeting' may be more beneficial for combatting disease.

Table of Contents

List of Tables viii
Acknowledgements x
Dedication xi
Chapter 1 - Literature Review 1
  Grafting 1
    Brief History of Vegetable Grafting 1
    Grafting and High Tunnel Production 2
    Grafting Technique 8
      Propagation 9
      Tube Grafting procedure 9
      Healing Chamber Management 10
    Physiology of graft union healing 11
  Rootstock-Scion Interactions 13
    Graft Compatibility 14
      Types of Graft Incompatibility 16
    Mechanisms of Incompatibility 18
      The Role of Auxins 20
      Genetic Basis 21
      Graft Hybridization 24
        Mentor Grafting 25
        Horizontal Gene Transfer 27
      Virus-Mediated Delayed Incompatibility 27
      Immune Response-Related Incompatibility 28
      “Graft Incompatibility Toxins” 28
    Overcoming Graft Incompatibility 29
  Research Introduction 31
Chapter 2 - Fruit Yield and Scion Compatibility of Ten Hybrid Determinate Tomato Varieties Grafted With ‘Maxifort’ Rootstock 32
  Summary 32

Introduction 33

Materials and Methods 40

- Transplant Production and Grafting 41
- Growing Methods 42
  - 2016 Trial 42
  - 2017 Trial 43
- Data Collection 43
- Data Analysis 44

Results 47

- Main and Combined Effects 47
- Impact of Scion Variety and Grafting on Fruit Yield 47
- Impact of Scion Variety and Grafting on Fruit Size 48
- Graft-Induced Changes in Marketable Yield 48
- Impact of Grafting and Scion Variety on Yield Ranking 49
- Impact of Grafting and Scion Variety on Plant Biomass, Relationship to Yield 49

Discussion 50

- Rootstock and Scion Relationship Dynamics 50
- Determining Rootstock and Scion Compatibility 53
- Successful Combinations for High Tunnel Production 57

Conclusions 58

Tables 61

Chapter 3 - Evaluating *Solanum* and *Capsicum* Rootstocks for Fresh-Market Bell Pepper Production 68

Summary 68

Introduction 70

- Pepper Grafting 72
- Compatibility 75
  - Predicting Compatibility Within the Solanaceous Family 76

Project Goals 79

Materials and Methods 80

- Transplant Production and Grafting 82

        - Olathe Horticulture Research and Extension Center (OHREC) 83
        - John C Pair Horticulture Center (JCPHC) 84
        - Willow Lake Student Farm 85
        - Butler County On-Farm Trial 86
        - Data Collection 87
        - Data Analysis 87
    - Results 90
        - Scarface 91
        - Yaocali 92
        - Meeting 93
        - Maxifort 93
        - Sweetie 94
    - Discussion 95
        - Grafting Bell Pepper for Increased Productivity 96
        - Intergeneric Grafting 97
    - Conclusions 100
    - Tables 102
- Bibliography 109

List of Tables

Table 2.1 - Disease resistance (as reported by seed companies) for determinate tomato scion and vegetative rootstock varieties used in a tomato scion grafting trial conducted in 2016 and 2017 in a high tunnel in Olathe, KS. Resistance codes based on (Johnny's Selected Seeds, 2018b) 61

Table 2.2 - *P*-values of main and interaction effects for: year, graft-status, and scion on marketable and total fruit yield for a tomato scion grafting trial conducted in 2016 and 2017 in a high tunnel in Olathe, KS. Data was transformed by year using the z-score standardization procedure prior to analysis. 62

Table 2.3 - Simple effects of scion and graft-status on marketable and total fruit yield and size for grafted and nongrafted tomatoes grown in a high tunnel in 2016 and 2017 in Olathe, KS. 63

Table 2.4 - Actual and percent change in marketable yield of ten tomato varieties due to grafting with 'Maxifort' rootstock when grown in a high tunnel in 2016 and 2017 in Olathe, KS. .. 64

Table 2.5 - Ranked mean values for marketable and total yield for ten grafted and nongrafted tomato varieties grown in a high tunnel in 2016 and 2017 in Olathe, KS. 65

Table 2.6 - Tukey's honest significant difference test for above and below-ground dry biomass as effected by graft status and scion variety for a tomato grafting trial conducted in 2017 in a high tunnel in Olathe, KS 66

Table 2.7 - Correlations between biomass and yield for a tomato grafting trial conducted in 2017 in a high tunnel in Olathe, KS (N=80) 67

Table 3.1 - Analysis of variance P-values for Main Effects: Trial and Rootstock as well as effect interactions for both marketable and total fruit yield, percent marketability, and both above and below-ground biomass for a sweet pepper rootstock grafting trial conducted in 2016 and 2017 in five locations in central and eastern Kansas. Data was transformed by trial using the z-score standardization procedure prior to analysis. 102

Table 3.2 - determination of appropriate statistical procedures for both marketable and total fruit yield, and percent marketability based on the results of Variance Homogeneity and Normality Tests, for a sweet pepper rootstock grafting trial conducted in 2016 and 2017 in

five locations in central and eastern Kansas. Data was transformed by trial using the z-score standardization procedure prior to analysis. 103

Table 3.3 - Marketable yield and fruit size for a sweet pepper rootstock grafting trial conducted in 2016 and 2017 in five locations in central and eastern Kansas. Data was transformed by trial using the z-score standardization procedure prior to analysis, and back transformed for presentation here. 104

Table 3.4 - Total yield and overall size for a sweet pepper rootstock grafting trial conducted in 2016 and 2017 in five locations in central and eastern Kansas. Data was transformed by trial using the z-score standardization procedure prior to analysis, and back transformed for presentation here. 105

Table 3.5 - Percent marketability by weight and number for a sweet pepper rootstock grafting trial conducted in 2016 and 2017 in five locations in central and eastern Kansas. Data was transformed by trial using the z-score standardization procedure prior to analysis, and back transformed for presentation here. 106

Table 3.6 - Pearson Correlation Coefficients for relationship between yield and plant morphological characteristics measured by plant height (ground level to the longest vertical meristem) and dry biomass for a sweet pepper rootstock grafting trial conducted in 2016 and 2017 in five locations in central and eastern Kansas (N = 60). 107

Table 3.7 - Above and Below-Ground Dry Biomass (g) for a sweet pepper rootstock grafting trial conducted in 2016 and 2017 in five locations in central and eastern Kansas. Data was transformed by trial using the z-score standardization procedure prior to analysis, and results were back-transformed for presentation. 108

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Dedication

I dedicate this work to the vine. Every time I was exhausted from a late night, was overwhelmed by the task ahead, or felt alone, you were there. You helped me to understand, gave me the strength to persevere, and provided the courage to move ahead into the unknown. Thank you.

"I am the vine; you are the branches. If you remain in me and I in you, you will bear much fruit; apart from me you can do nothing."

John 15:5 (NIV)

Chapter 1 - Literature Review

Grafting

Brief History of Vegetable Grafting

The practice of grafting is ancient, and likely originated after people observed naturally grafted or "inosculated" plants. Greek, Roman, and Chinese sources indicate that grafting was a common practice as early as the fifth century BCE (Melnyk and Meyerowitz, 2015; Mudge et al., 2009), and in the first century CE, the apostle Paul makes multiple references to the grafting of improved cultivars of olive trees onto wild rootstocks (Romans 11:17-24). References to herbaceous grafting (involving watermelon) are found in 5th century Chinese literature and in Korean writings from the 1600s (Kubota et al., 2008). However, the first record of solanaceous grafting seems to involve the work of James Edward Rice, an undergraduate student at Cornell University who, in 1890 attempted a wide variety of interspecific (between different species of the same genus) and intergeneric grafts (between species of different genera, but typically within the same family). Rice attempted to graft coleus, beans, cucurbits, and a variety of solanaceous plants including peppers, eggplant, tomato, and potato, for which he reported a good degree of interspecific and intergeneric compatibility (Rice, 1891). The grafting of melons became popular as a method for controlling soilborne disease between the 1920s and 1930s (Kubota et al., 2008) and around this time the grafting of tomato onto jimsonweed was growing in popularity in the southern United States, as it was found to be an effective way to address problems caused by root-knot nematode (Kubota et al., 2008; Lowman and Kelly, 1946; Sen et al., 2018) Such grafting soon fell out of favor when it was determined that alkaloids from the roots could migrate to the fruit in quantities that could potentially prove harmful if consumed (Lowman and Kelly, 1946). However, by the 1960s, the development of vigorous and disease resistant interspecific hybrid rootstocks (typically hybrids between wild and cultivated species within the same genus) was beginning. Rootstocks like ‘KK’, De Ruiter’s first ever tomato rootstock, released in 1963, provided resistance to nematodes and corky root rot, and other rootstocks with verticillium and fusarium wilt resistance soon followed (DeRuiter, 2013). By the 1970s, solanaceous grafting was once again becoming popular, especially in Japan and Korea, and since the early 1990s the practice has grown significantly in popularity in Asia, Europe and the United States (Kubota et al., 2008).

Grafting and High Tunnel Production

High tunnels, or “hoop houses” are unheated, greenhouse-like structures typically enclosed with polyethylene plastic, providing a relatively inexpensive method for passive climate control (Buller et al., 2016; High Tunnels.org, 2010). For this reason, they have made the advantages of season-extension technology available for those of more modest means and on a larger scale than ever before possible (Blomgren and Frisch, 2007; Kaiser and Ernst, 2012; Lamont, 2009; Thompson, 2012). The season extension provided by a high tunnel makes them particularly attractive to growers marketing their produce locally (Gent, 1991; Hunter, 2010; Kaiser and Ernst, 2012; Knewtson, 2008), and the resulting improvements in yield over the open-field (Altamimi, 2010; Blomgren and Frisch, 2007; Jensen and Malter, 1995; Palonen et al., 2017) make them very popular for small to medium-scale, urban or peri-urban fruit and vegetable producers, for whom space may be limited (Jensen and Malter, 1995). The increased awareness of both their effectiveness and the relatively low initial investment has helped the popularity of high tunnels to grow substantially since the early 1990s (Carey et al., 2009; Frey et al., 2017; Huff, 2015).

High tunnels are especially popular among producers of solanaceous crops like tomatoes (*Solanum lycopersicum*) and peppers (*Capsicum annum*), which have become the most prolific and profitable warm-season crops grown in these structures (Buller et al., 2016; Carey et al., 2009; Edelstein, 2004; Galinato and Miles, 2013; Goldy, 2012; Knewtson, 2008; Lamont, 2009; Reeve and Drost, 2012; Wright, 2016). By increasing the total number of growing-degree days, and maintaining a higher soil temperature at both ends of the summer growing season (Gu, 2017; Knewtson, 2008), the use of a high tunnel may advance the grower's production system by up to one and a half plant hardiness zones (Gu, 2017), allowing for a planting date as much as 30 days in advance of the open-field, (Gu, 2017; Reeve and Drost, 2012; Thompson, 2012). This increase in the number of growing-degree days accelerates the growth, development and ripening process of solanaceous crops (Gent, 1991) and may lead to a harvest between 2 and 5 weeks in advance of open-field crops (Gent, 1991; Kaiser and Ernst, 2012; Thompson, 2012). The high tunnel microclimate can also extend the growing season on the late end, which can lead to crop production well past the close of the open-field season (Buller et al., 2016; Gu, 2017; Lamont, 2005; Thompson, 2012).

The physical barrier provided by high tunnels protects the plants within from climactic stressors such as rain, wind, and hail (Reeve and Drost, 2012). By excluding rain, the high tunnel barrier helps to avoid or at least inhibit the spread of (primarily bacterial) diseases associated with foliar moisture (Altamimi, 2010; Blomgren and Frisch, 2007; Hardwick, 2002; Kaiser and Ernst, 2012; Knewtson, 2008). The reduction in air movement associated with covered agriculture can also reduce the spread of certain (especially fungal) diseases (Hardwick, 2002). While overall plant stress and disease pressure in high tunnels is reduced (Altamimi, 2010; O'Connell et al., 2012), leading to improvements in crop quality (Blomgren and Frisch, 2007;

Huff, 2015; Lamont, 2005; O'Connell et al., 2012), the use of high tunnels is not without problems.

Because of the productivity (and profitability) of high tunnels, producers with limited space (especially in urban areas) are often reluctant to allow such a valuable production area to sit fallow, and many producers either completely ignore crop rotation, or use shorter, less effective rotation intervals (Buller et al., 2016; Goldy, 2012). For this reason, soilborne diseases involving the buildup of pathogen inoculum (Kaiser and Ernst, 2012; Knewtson, 2008), viruses (Kaiser and Ernst, 2012), and parasites such as root-knot nematodes, which are typically managed by crop rotation (Guan, 2017), are common problems in high tunnels. The intensively cropped and therefore frequently tilled nature of high tunnels can cause soil nutrient depletion (Reeve and Drost, 2012), and drip irrigation, rain exclusion and the absence of leaching that occurs when soil remains covered for long periods can lead to increased soil salinity or calcium deficiencies (Altamimi, 2010; Blomgren and Frisch, 2007; Kaiser and Ernst, 2012; Knewtson, 2008).

The implementation of crop rotation is a persistent challenge for covered agriculture (Oda, 1999), and (highly profitable) monocropped solanaceous plants (typically tomatoes and peppers) continue to dominate the high tunnel landscape (Buller et al., 2016; Carey et al., 2009; Edelstein, 2004; Galinato and Miles, 2013; Goldy, 2012; Knewtson, 2008; Lamont, 2009; Reeve and Drost, 2012; Wright, 2016). Given the economic importance of these crops (Lin and Morrison, 2017), this trend is likely to continue. As a result, and partially because of the phase-out of many chemical soil fumigants, a growing number of producers, both worldwide and increasingly in the U.S. (Kubota et al, 2008), are utilizing grafted tomato and pepper plants in an attempt to improve productivity and address problems common to covered agriculture (Albacete et al., 2015; Louws et al., 2010; Michel et al., 2015; Rivard and Louws, 2011)

The primary advantages of grafted plants can be divided into three main categories: 1) improving tolerance/resistance to soilborne diseases and nematodes, 2) increasing yield and percent marketability, and 3) improving plant tolerance of harsh growing conditions, especially soil salinity and waterlogged soils (Kubota et al., 2008). Grafted plants have been shown to produce higher yields (Bletsos and Olympios, 2008; Davis et al., 2008b; Djidonou et al., 2013; Edelstein, 2004), when certain rootstocks are utilized and especially when used in covered production systems. This is not only because they are better able to tolerate the drawbacks of such a covered system (Buller et al., 2016; Giles, 2011; Rodriquez and Bosland, 2010), but also because they seem to be better able to take advantage of the improvements offered by a high tunnel growing environment than their nongrafted counterparts, factors that result in improved productivity in terms of both percent marketability and fruit quantity (Davis et al., 2008b; Haberal et al., 2016; Loewen and Rivard, 2016; Loewen et al., 2016). While some studies have also demonstrated improvements in yield under open-field conditions (Djidonou et al., 2013), the application of grafted plants merely to improve yield is probably more advantageous for growers utilizing covered agriculture than for those with an open-field growing system, unless the grower also wishes to address problems of soil-borne disease or abiotic stress.

Grafting can improve productivity in a variety of ways (Masterson et al., 2016; Rivard and Louws, 2008). First, grafting improves the overall health of the plant (Albacete et al., 2015). Grafted plants on highly vigorous rootstocks are more efficient at moving water and nutrients, have larger root systems, and have greater vegetative production (Djidonou et al., 2013; Haberal et al., 2016; Lee, 1994), perhaps due to the production of endogenous plant hormones or other proteins in the rootstock. In many graft relationships, phloem proteins (P-proteins) mediate the movement of these proteins and hormones to the scion (Golecki et al., 1998), where they direct its growth and development (Edelstein, 2004; Lee, 1994). In addition to plant hormones and proteins, mRNAs (Lucas et al., 2001), including those involved in apical meristem development (Ruiz-Medrano et al., 1999), entire plastid genomes (especially Mitochondria) (Gurdon et al., 2016), and in some cases entire nuclear genomes, can also cross the graft barrier via the phloem and be expressed in the scion (Fuentes et al., 2014), altering its physiology. This physiological alteration has been shown to occur even in intergeneric graft unions (Haberal et al., 2016).

However, alterations in scion physiology vary significantly depending on the specific rootstock/scion combination and may or may not be favorable. For example, tomato scion grafted onto tobacco rootstock displayed higher growth and leafing, earlier flower and fruit set, and significant increases in yield over nongrafted tomato plants (Haberal et al., 2016). On the other hand, the use of a dwarf tomato as a rootstock also dwarfed the scion it was grafted to, while a standard rootstock resulted in normal growth when paired with the scion of a dwarf variety, an action that was linked to the transfer of mRNAs (Albacete et al., 2015). Changes in leaf and flower physiology were also observed with a variety of interspecific and intergeneric grafts including potato scion on tomato rootstock (Kudo and Harada, 2007), eggplant scion on tomato rootstock, and pepper scion on tomato rootstock, and were attributed to mRNA and protein transfer from the rootstock to the scion (Eltayb et al., 2013).

The improved overall plant vigor associated with well-chosen graft partners helps to make the plant more tolerant of pests, disease, and environmental stressors as well as improving the potential of the plant to produce high yields (Blomgren and Frisch, 2007; Lee, 1994; Loewen and Rivard, 2016). Such vigor and disease tolerance observed when grafting with a well-chosen rootstock, can function to improve the percent marketability of the fruit produced as well (Edelstein, 2004). However, such improvements in fruit are inconsistent, and depend heavily on the interactions taking between specific rootstock/scion pairs as well as the environment in which the produce is grown (Davis et al., 2008b). It is therefore important to consider the suitability of grafted plants for a specific (in this case high tunnel) production system not merely in terms of Genotype x Environment, but also in terms of Rootstock x Scion x Environment (Albacete et al., 2015).

In addition to disease tolerance resulting from improved overall vigor, many rootstocks, beginning with melons and gourds in the 1920s have been selected for disease resistance (Davis et al., 2008a; Rodriquez and Bosland, 2010) or hybridized with local wild relatives (if available) which likely already have resistance to common diseases in the area (Albacete et al., 2015). Such traits are highly valued, especially in Southeast Asia where soilborne diseases like bacterial wilt can cause total tomato crop losses (Schreinemachers and Afari-Sefa, 2012). Solanaceous rootstocks are now available which are resistant to a variety of soilborne pathogens including: nematodes, fusarium, verticillium, and bacterial wilts (Bletsos and Olympios, 2008; Blomgren and Frisch, 2007; Edelstein, 2004; Fernández-García et al., 2004; Lee, 1994; Rivard and Louws, 2006; ‘Tomato Grafting’, 2015). While most of these varieties exhibit genetic “non-host” resistance, some disease-resistance may be rootstock induced systemic resistance. Such systemic resistance functions via mRNA or hormonal signaling, much of which is related to stress and defense responses (Goldschmidt, 2014; Omid et al., 2007), and may also involve the production of immune proteins, allowing for a degree of resistance even when the scion develops an adventitious root system (Lee, 1994). However, it should be noted that genetic resistance may break down under less than ideal growing temperatures (Fraser and Loughlin, 1982).

Additionally, whereas some resistance genes are very robust, others may be overcome by pathogens after as few as three successive seasons of planting using the same resistance gene profile (Ros-Ibáñez et al., 2014).

Some rootstocks also help plants to tolerate abiotic stress. Such stress is caused by detrimental environmental conditions (Edelstein, 2004; Rivard and Louws, 2006; Sen et al., 2018; Wu et al., 2012), nutrient imbalances or deficiencies which are non-living and non-infectious by nature, but which, in addition to compromising the overall health of the plant also compromise its ability to combat disease. Most abiotic stress tolerance is directly related to the physiology and tolerances inherent to the rootstock. These include salt tolerance in some tobacco rootstocks (Haberal et al., 2016) , low soil-temperature tolerance found in certain *Solanum* rootstocks (Bletsos and Olympios, 2008; Edelstein, 2004), and waterlogging tolerance in a variety of eggplant (especially wild varieties) and chili pepper rootstocks (Bahadur et al., 2015; Petran, 2013; Schreinemachers and Afari-Sefa, 2012; 'Tomato Grafting', 2015; Wu et al., 2012).

Grafting Technique

Prior to the turn of the 21st century, the most common technique for solanaceous grafting was cleft grafting, a process which involves cutting the stem of the scion into a wedge and fitting it into a cleft cut into the rootstock (Oda, 1999; Singh, 1949). However, since its development in 1996 (DeRuiter, 2013), the "Tube-grafting" or "Japanese top-grafting" method has become very popular for grafting solanaceous crops. The popularity of tube-grafting as compared to cleft and approach grafts is due to its high success rate, the relative simplicity of the process, the reduced plant size required for grafting, the improved strength of the healed graft union, a reduced propensity for adventitious rooting, and the improved speed (as much as three times that of cleft grafting) with which high numbers of plants can be grafted and managed through the healing process (McAvoy, 2005; Oda, 1999; Petran, 2013; Rivard and Louws, 2006). Grafting smaller (younger) plants improves space efficiency and may also increase the speed with which the vascular tissue reconnects, further improving the overall grafting success rate (Johkan et al., 2009; Petran, 2013).

Propagation

The splice grafting process takes approximately 6-8 weeks from sowing seed to having field-ready transplants, and should therefore be started approximately two weeks prior to typical non-grafted transplant production (Rivard and Louws, 2006; Rivard et al., 2010a). Matching the stem diameters of scion and rootstock when grafting is of the utmost importance for encouraging the effective reconnection of the vascular bundles through the graft union, improving the grafts strength (Andrews and Marquez, 1993), and reduce the incidence of graft union deformities (Hartmann et al., 2013a). Because both the time to germination and the growth rate for (often interspecific hybrid) rootstock and scion cultivars frequently differ, it is advisable to plant a few seeds of both varieties early in the season to approximate the necessary seeding-date offset (if any). If after seeding, the stems do not match up, or it becomes apparent that one variety is growing more rapidly than another, the faster growing variety may be held back by placing it in a cooler growing environment (MacDonald, 2014; McAvoy, 2005; Rivard and Louws, 2006).

Tube Grafting procedure

When using the tube-grafting method, plants may be grafted as early as 14-21 days. This is typically when the stems are between 2 and 3 mm in diameter and have 1-2 pairs of true leaves. Immediately prior to grafting, the grafter should consider removing between 80 and 90% of the leaves from the scion variety (being careful to leave the apical meristem intact). Such leaf removal has been shown to significantly reduce adventitious root formation (Meyer et al.,2017) and increase grafting success (Masterson et al., 2016). To graft, both rootstock and scion are cut at the same approximately 70° angle from horizontal (Bausher, 2013) using a sharp, sterile blade, and are held together snugly with a silicon grafting clip (MacDonald, 2014; Masterson et al., 2016a; McAvoy, 2005; Meyer et al., 2017 Oda, 1999; Rivard and Louws, 2006).

Healing Chamber Management

Once grafting is complete, the plants are placed into a healing chamber, where temperature, light, and humidity are controlled for the next 7-10 days to prevent the plants from becoming water-stressed while the callus forms and the vascular tissue reconnects (Rivard and Louws, 2006). Proper management through the healing process is potentially more critical to the success of a grafting endeavor than the actual act of grafting (Hartmann et al., 2013a). For the first 3-4 days, the relative humidity of the chamber is maintained at over 90%, with the temperature between 70 and 80°F. It is common for the scion tissue to wilt during this period, though prior leaf removal and high humidity should help maintain some turgidity in the scion (Rivard and Louws, 2006). The high humidity during these days also helps to keep the callus tissue in a state that is conducive to graft healing by preventing the formation of a suberized periderm on the outer layer of the callus masses (Moore, 1984). Once wilting has resolved (typically by day 4) the humidity within the chamber is gradually reduced, and light is increased so that by day 7-10, the plants will tolerate ambient conditions (MacDonald, 2014; Rivard and Louws, 2006). This healing process is typically moved along as rapidly as is reasonable, since leaving the plants in a low-light, high humidity environment can cause problems with edema (Rivard and Louws, 2006) and etiolation (stretching), and increases the risk for fungal and bacterial infections, pathogens which thrive in the dark, warm, and humid conditions of the healing chamber (MacDonald, 2014), for this reason, good sanitation throughout the grafting and healing process is important (Rivard and Louws, 2006).

Physiology of graft union healing

The basic formation of the graft union involves commandeering the plants natural wound-healing response in order to get the two halves of the new plant to heal together (Andrews and Marquez, 1993; Gainza, Opazo and Muñoz, 2015; Moore, 1984; Tamilselvi and Pugalendhi, 2017; Yin et al., 2012). However, it has been shown in Arabidopsis that certain markers whose upregulation is associated with the grafting process in both the rootstock and scion are upregulated in the shoot, but not in cut roots as a part of a normal wounding response. This indicates that the presence of the scion influences the response seen in the rootstock, and suggests that the graft healing process may be a more complex and cooperative effort than a basic wounding response (Melnyk et al., 2015). Some have even suggested that wounding prior to grafting, though it will not directly cause graft incompatibility, may serve to increase the severity of such symptoms (Andrews and Marquez, 1993).

The graft healing process occurs in a basic sequence of events (Gainza, Opazo and Muñoz, 2015). After cutting, the damaged cells collapse and form necrotic layers on the cut surfaces of both the rootstock and scion (Fernández-García et al., 2004; Melnyk et al., 2015). These necrotic layers then adhere to one-another, likely as a result of the deposition and polymerization of cell wall materials from the damaged tissues (Moore, 1984). In successful grafts, cell-to-cell communication is established early on, even while the healthy cells around the wound are still cleaning up cell debris in the necrotic tissue layer caused by the grafting process (Yin et al., 2012). Within about four days, a mass of pluripotent callus cells has formed by the hypertrophy and proliferation of both cambial and parenchymatous cells near the graft union.

The callus masses of both stock and scion meet and interlock, forming the graft interface (Andrews and Marquez, 1993; Fernández-García et al., 2004; Melnyk et al., 2015; Moore, 1984).

Finally, vascular re-differentiation of the callus parenchyma cells across the graft interface is initiated by auxins exuded by the cut ends of the scion's vascular cambium, (Gainza et al., 2015; Goldschmidt, 2014; Melnyk et al., 2015; Moore, 1984; Yin et al., 2012). The buildup of auxins around the graft union begins early on in the healing process, and may be detected as little as 48 hours after grafting (Gainza et al., 2015). This auxin buildup has been implicated in the formation of problematic adventitious roots (Maldiney et al., 1986; Meyer et al., 2017). Some have suggested that peroxidase and catalase may also be involved in this process, because peroxidase catalyzes the lignification of the developing xylem, and levels of both Peroxidase and Catalase increase around the graft union following grafting (Fernández-García et al., 2004). However, both catalysts, and catalase in particular, function to reduce harmful levels of the reactive oxygen species H_2O_2 (Hydrogen Peroxide). It therefore seems likely that the presence of these catalysts around the graft union may be in response to increased levels of H_2O_2, produced as a part of the general wound-induced stress response, and may not be directly related to the graft-healing process (Fernández-García et al., 2004; Slesak et al., 2007). This re-differentiation may begin as early as the fourth day after grafting and, depending on the compatibility of the grafting partners, the skill of the grafter, and the conditions provided for graft healing, the primary vascular connections (in a tomato graft) may be functional by between the fifth and seventh day after grafting. Though the graft is considered functional at this point, it is not fully developed until around the fifteenth day after grafting (Fernández-García et al., 2004; Turquois and Malone, 1996). Based on studies of Arabidopsis models, this new cambium gives rise to phloem first and then xylem, though the xylem likely forms in the scion tissue slightly prior to its formation in the root stock tissue (Melnyk et al., 2015). This is likely due to the differential influence of a gradually increasing flow of auxins from the scion, as lower auxin levels initiate cambium differentiation into phloem while higher levels initiate cambium differentiation into xylem (Shimomura and Fuzihara, 1977).

Rootstock-Scion Interactions

Environmental conditions can have a substantial effect on the performance of a grafted plant. Grafting creates a single organism with a composite genotype, and many grafting studies report significant environmental interactions (Soltan et al., 2017). For this reason, a simple Genotype by Environment (GxE) approach is inadequate when examining grafted plants because there are interactions between the composite genotypes of the organism which occur in addition to, and not entirely independent of, each genotypes interaction with the environment. While significantly more complex, a Rootstock x Scion x Environment approach is more useful (Albacete et al., 2015, Leal-Fernández et al., 2013). Generally, GxE studies will include trials under multiple environments to discover interactions which might not be expressed under a single set of growing conditions. However, when studying grafting, controlling for at one of the three factors helps considerably in understanding the relationship between the other two. For this reason, it is common to choose a small number of “good” environments, which provide a high yield for most varieties of the crop in question and are analogous to the ideal (or target) growing environment. Obviously, different criteria may be applied for studies looking at resistance to/tolerance of disease, pests or abiotic stressors. This careful environmental selection allows for a more accurate genotype-to-genotype assessment, with more easily discernable differences in compatibility and productivity (Hayward et al., 1993). A high tunnel growing environment is therefore useful for grafting research, both to replicate the target growing environment of the study and to help tease out any differences created by the Rootstock x Scion interactions.

Graft Compatibility

Grafting is, at its' core, very similar to the production of hybrids, as it is an attempt to take advantage of the interactions between two different genotypes. In highly compatible graft pairings, the growth response of the scion to a vigorous rootstock seems to mirror the enhanced growth or "hybrid vigor" observed in many hybrid cultivars. This suggests that the interaction of two different, but related genotypes (whether via hybridization or grafting) may provide an invigorating effect on the whole plant (though the scion may benefit the most, as many rootstocks already benefit from hybrid vigor) (Albacete et al., 2015). When grafting, "compatibility", involving these interactions between genotypes and the degree to which they are beneficial, becomes critically important (Tamilselvi and Pugalendhi, 2017).

Unfortunately, many graft combinations which would likely be desirable due to specific characteristics available in different rootstocks and scions are considered incompatible (Andrews and Marquez, 1993). Graft compatibility is not entirely understood and appears to be a complex and multi-faceted issue with a variety of causes. For this reason, the case-by-case identification of particular compatibility issues often involves a certain degree of speculation (Gainza, et al., 2015; Goldschmidt, 2014), and may not be limited to the interactions between rootstock and scion. The environment often presents a third factor in the grafting relationship which can affect the communication and interaction between the grafting partners (Albacete et al., 2015).

Grafting (in)compatibility is often loosely defined as the failure or success of two plants, when grafted together, to survive and form a "successful" graft union (Kawaguchi et al., 2008; Tamilselvi and Pugalendhi, 2017). While this is essentially correct, there is a risk of prematurely declaring graft partners compatible. The initial graft-healing process, as important to the ultimate success of the graft as it is, does not always correlate with the long-term survivability or overall health of either the graft union or the new composite organism (Goldschmidt, 2014). A more precise and comprehensive definition of the term is therefore required.

Goldschmidt, (2014) outlined three basic characteristics of compatible grafting partners: 1) the establishment of a successful graft union 2) the extended survival of the composite plant, and 3) the proper functioning of the composite, grafted plant. Edelstein (2004) and Gainza et al.,(2015) elaborated on these concepts when describing basic symptoms of incompatibility. These symptoms included a failure of the rootstock and scion to unite, a lack of health, graft breakage or failure of the graft union, the premature death of the plant, and the inability for the grafting partners to form a strong, lasting, and functional union. Combined, these concepts provide a more precise three-part definition. Graft compatibility entails the relative capacity of two grafting partners for the following: 1) the establishment and proper healing of a strong and effective graft union, 2) the extended survival of the composite plant, indicated by a lifespan typical to, or greater than others of the same species, and where the death of the composite organism is not directly related to the late-stage failure of the graft union, and 3) the general health and overall productivity of the composite plant. A failure to meet any of these requirements could be indicative of a compatibility issue.

Given the list of characteristics involved, compatibility can be described as a spectrum, with many grafting combinations falling somewhere between wholly incompatible and highly compatible (Oda, et al., 2005). Even within a particular species, grafting compatibility is often very specific, meaning that a particular rootstock will likely not be equally compatible with all cultivars of a given species (Gainza et al., 2015). Therefore, choosing specific and proven rootstock/scion combinations is important, since what works well for with one cultivar or rootstock variety may give very different results when paired to a different cultivar.

It should be noted that while graft failure is often tied to incompatibility, there are many other reasons a graft may fail. Simply because a graft fails to heal initially or breaks soon afterwards does not mean that the rootstock and scion in question are necessarily incompatible (Andrews and Marquez, 1993; Tamilselvi and Pugalendhi, 2017). The failure of a graft union may be caused by a variety of factors including less-than-ideal environmental conditions, infection, and poor grafting technique (Andrews and Marquez, 1993; Tamilselvi and Pugalendhi, 2017).

Types of Graft Incompatibility

Graft incompatibility is typically considered either immediate or delayed, however, it should be noted that not all incompatibility will manifest in the same manner, and there are some cases where two species or cultivars may be considered only “moderately incompatible”. For example, moderate graft incompatibility was observed in inter-specific eggplant/tomato grafts (Goldschmidt, 2014; Kawaguchi et al., 2008; Oda et al., 2005). This incompatibility was characterized by reductions in fruit yield and percent marketability and was attributed to differences in the nutrient requirements between eggplant and tomato (Kawaguchi et al., 2008), specifically the low Ca concentrations observed in the scion of such grafts by Oda et al., (2005). Since the vascular connections appeared to be relatively normal (Kawaguchi et al., 2008) such graft pairings may still be useful under certain conditions, as in cases where severely waterlogged soils create a significant barrier for the production of tomatoes unless they are grafted onto eggplant rootstocks (Bahadur et al., 2015). A similar Ca deficiency was also noted in tomatoes grafted onto other rootstock species, as well as deficiencies in Mo and P in cucumber/pumpkin heterografts, and for Mg in melons grafted onto cucurbit rootstocks (Oda et al., 2005).

Immediate graft incompatibility is typically expressed shortly after grafting, is characterized by the rapid death of the scion tissue, and is attributed to the failure of the vascular tissue to heal and function properly (Mudge, 2013). At times however, the degree of compatibility displayed by grafting partners may appear to change as the composite organism develops. The phenomena, known as "delayed incompatibility" is typically characterized early on by the apparent formation of a successful graft union (Andrews and Marquez, 1993), and only in later development or maturity displays obvious symptoms of incompatibility. In delayed incompatibility these symptoms are often visible external deformities or necrosis at the graft interface which are associated with vascular discontinuity and other vascular irregularities. In woody plants, such delayed incompatibility may only manifest itself after several years of apparent graft health (Gainza et al., 2015 ; Mudge, 2013). It has been suggested that such delayed incompatibility may not actually be delayed, but may begin shortly after the formation of the graft union, and progress for a time without causing any symptoms (Mudge, 2013). Though typically a frustrating development, some have suggested that delayed incompatibility may be used to help root cuttings. In theory this would work by grafting a desirable scion onto a known incompatible rootstock and planting the composite plant with the graft union below ground level. Ideally, the scion will have time to form its own roots prior to the failure of the graft (Hartmann et al., 2013a).

In studies with woody plants, the over or under-growth of the scion creates a graft union with an abnormal appearance. Such discrepancies in the growth of graft partners are thought to correlate with the degree of compatibility between graft partners. Anatomical explorations of such grafts reveal degrees of vascular irregularity and interference with the flow of water and nutrients typically consistent with the external observations (Gainza et al., 2015). Because the survival of the scion tissue and therefore the success or failure of a graft union is largely dependent on the rapid reestablishment and proper functioning of the vascular connection between the rootstock and scion (Gainza et al., 2015), irregularities in the vascular bundles at the graft union (including a reduced number or complete lack thereof) appear to be a major contributing factor in both immediate and delayed incompatibility. This is because the irregularities and discontinuities observed in the vascular tissue greatly reduce the hydraulic conductivity at the graft union, inhibiting the transport of water, minerals, and nutrients between stock and scion (Goldschmidt, 2014; Kawaguchi et al., 2008; Oda et al., 2005). Such vascular discontinuities have been observed at the graft interface of incompatible pepper/tomato graft unions. These irregularities have been implicated in incompatibilities in pepper/tomato grafts and their reciprocals, characterized by growth inhibition, high leaf chlorophyll content, the production of undersized fruit, wilting, and high rates of mortality (Kawaguchi et al., 2008; Oda et al., 2005).

However, as mentioned previously, compatibility exists in a spectrum and may be negligible or severe, depending on the degree of vascular deformity that occurs at the graft union (Oda et al., 2005). As such, external graft union “deformities” do not always indicate a lack of compatibility, as some grafting combinations show no symptoms of incompatibility besides possessing a union with an irregular outward appearance (Bitters, 1986; Goldschmidt, 2014).

Mechanisms of Incompatibility

The mechanisms behind some major rootstock/scion interactions seem to be clear, i.e. the dwarfing or enhanced scion growth observed when using certain rootstocks is often understood as a simple result of either enhanced or reduced water and nutrient uptake by a more or less vigorous root system (Albacete et al., 2015; Goldschmidt, 2014). However, a subtler and more complex relationship also exists that can affect the compatibility, the growth, and the physiology of the grafting partner.

This relationship involves the movement of not only water and nutrients through the graft union, but also requires intercellular recognition, coordination, and communication between the tissues of the grafting partner for the formation of a functional graft union (Andrews and Marquez, 1993). This whole-plant communication network is generally bidirectional through the vascular tissue (and therefore through the graft interface) and includes the exchange of hormones, metabolites, peptides, small organic molecules, and macromolecules such as proteins and nucleic acids (specifically mRNA and other small RNA signals) (Albacete et al., 2015; Goldschmidt, 2014). These signals are believed to play an important role in regulating the use of nutrients and the physiological development of grafted plants. In this way both the scion and rootstock may affect developmental changes on their grafting partner (Albacete et al., 2015; Goldschmidt, 2014). Plasmodesmata serve to regulate the movement of these signals through the vascular tissue of the plant, and also play an important, if more localized, role in intercellular recognition and communication between the graft partners at the graft interface itself. The plasmodesmata may even facilitate such intercellular communication and coordination as early as the differentiation of the callus tissue, and likely contribute to the proper functioning of the graft, as mismatched and partially formed plasmodesmata have been observed in incompatible heterografts (Pina and Errea, 2005).

The Role of Auxins

While it is recommended that the vascular tissues of the grafting partners be well aligned to promote a strong graft union, and the goal of most grafting is for direct cambial contact between rootstock and scion (Hartmann et al., 2013a), neither a lack of direct vascular tissue contact nor even a degree of anatomical discrepancy between grafting partners will prevent the formation of a successful graft union (Andrews and Marquez, 1993). It has even been shown that the coordination necessary for callus re-differentiation will still occur when the stock and scion are separated by a porous membrane filter which prevents direct cellular contact without impeding the flow of diffusible substances (Moore, 1984). Conceptually, this would seem to point to hormones such as auxins, rather than to direct cellular contact as drivers of stock-scion recognition and graft healing.

Although it is likely that auxins are important in the reformation of the vascular tissue across the graft union, it is unclear how significant a role they play in rootstock/scion compatibility. In many incompatible grafts, the callus forms but fails to differentiate properly (Gainza et al., 2015). As auxins are not species-specific their function as directors of callus differentiation does not explain the degrees of compatibility that are often observed in various graft pairings (Abidin and Metali, 2015; Moore, 1984). Even in incompatible graft unions, vascular differentiation and stock to scion continuity in the callus tissue is often not initially inhibited (Gainza et al., 2015; Moore, 1984; Pina and Errea, 2005), though there appears to be a change in the pattern of vascular tissue differentiation. This suggests that in incompatible graft unions, auxins are still at work as a director of vascular tissue formation (and the formation of such vascular tissue in general). Though without the additional direction of other growth regulators, auxins may not be the ultimate deciding factor in the formation of a compatible graft union.(Andrews and Marquez, 1993). Therefore, an additional mechanism or set of mechanisms must be responsible for the varying degrees of graft compatibility observed.

Some auxins like indoleacetic acid are strongly associated with certain phenolic compounds (Bastin, 1966). These phenolic compounds are involved in the stress and wounding response and are released by both graft partners as a part of the grafting response (Gainza et al., 2015). In incompatible interspecific heterografts, there are often differences in both quantity and type of these compounds, and such differences may disrupt the cell cycle of the graft partner, inhibiting the formation and differentiation of the callus tissue, and leading to immediate graft incompatibility (Andrews and Marquez, 1993; Gainza et al., 2015). While the presence of auxins serves to accelerate the process of healing in compatible grafts, it may also be indirectly associated with the eventual breakdown of incompatible graft unions.

Genetic Basis

While incompatibility can be difficult to predict, in most cases graft compatibility appears to be a genetic issue, and therefore phylogenetic or taxonomic proximity is generally considered the best predictor of graft success. Therefore, as with attempts at hybridization, the initial, compatibility-related consideration when attempting to create a new, composite organism is typically phylogenetic proximity. The general trend is therefore as follows, homografts or autografts (grafting a plant back to itself or to another individual of the same cultivar) are presumably always compatible, and intraspecific grafts (between individuals of the same species, but often of different cultivars) are nearly always compatible. Interspecific grafts (between different species within the same genera) are frequently compatible, though incompatibility is not uncommon, while intergeneric grafts (grafts between species of different genres) are rarely compatible. Finally, interfamilial grafts (grafts between species of different taxonomic families) are nearly always incompatible, and there is very little compatibility to be found beyond this (Gainza et al., 2015; Goldschmidt, 2014; Hartmann et al., 2013a).

While these trends are generally predictive of compatibility, they are also imprecise. Compatibility is highly variable, and is thought to be controlled by multiple, interacting genes, making the specific factors at work difficult to identify (Andrews and Marquez, 1993; Venema et al., 2011). As such, significant differences in compatibility have been observed between cultivars of the same species on the same rootstock, and in some cases even in the reciprocal of a graft pairing (Dogra et al., 2018; Goldschmidt, 2014; Mudge, 2013).

For this reason, the role of genetic material in coordinating the successful pairing of graft partners can hardly be understated. There is evidence that much of the whole-plant intercellular communication network is gene-based, and RNA has been implicated as a prime mover in this network. It has also been demonstrated that RNA is well protected and stabilized within the phloem sap, where its movement is controlled via plasmodesmata and may be mediated by phloem proteins (P-proteins) (Lucas and Lee, 2004; Lucas et al., 2001; Ruiz-Medrano et al., 1999).

RNA is not only moved through the graft interface via the vascular system, it is also frequently expressed in the grafting partner. For example, potato scion grafted onto tomato rootstock showed altered leaf morphology in new growth (which is consistent with typical patterns of phloem-mediated viral movement). The presence of rootstock-specific mRNA transcripts was confirmed in the altered leaves (Kudo and Harada, 2007). Similar experiments with tobacco (N. tabacum) have also demonstrated the ability of graft transmitted mRNAs to silence the expression of certain traits in the scion (Zhang et al., 2014). Other mRNA have been shown to exert some control over scion development (specifically the development of the apical meristem) in intergeneric pumpkin/cucumber grafts (Ruiz-Medrano et al., 1999). Such RNA has even been implicated in the graft-mediated transferal of tolerance to abiotic stress (in this case the transfer of tolerance to continuous light from a grafted scion to shoots arising from intolerant rootstock tissues) (Velez-Ramirez et al., 2014), as well as virus and herbicide resistance (Albacete et al., 2015) indicating that such RNA signaling has a whole-plant effect. Mobile genetic expression likely also plays a role in the seemingly basic mechanisms involved in dwarfing rootstocks. Reciprocal grafting experiments with a dwarf tomato cultivar showed that not only could it dwarf a regular scion when used as a rootstock, but it could also be invigorated by a standard variety when used as the scion. This dwarfing and invigorating was related to the movement of certain mRNAs from stock to scion and their subsequent expression, indicating a basis for the physiological effects of a rootstock on its scion that involve not only differences in water and nutrient uptake, but a mobile gene expression component as well (Albacete et al., 2015). It seems that mRNA may be translated upon arrival at the target cell, or may regulate the transcription of other genes within the target cells native genome via DNA methylation (Lewsey et al., 2016; Ruiz-Medrano et al., 1999; Wu et al., 2013).

In studies with grafted melons, it has been noted that many of the mRNA transcripts transported between stock and scion were related to phloem structure and stress response (Omid et al., 2007) and may directly contribute to graft compatibility by helping to coordinate the re-vascularization of the callus tissue (in addition to Auxins). Other mRNAs are also believed to help coordinate vascular tissue development via ribonucleic protein (a protein-RNA complex) (Lucas et al., 2001), and some mRNA transcripts have been shown to be related to signal transduction, specifically for auxins, known drivers of cellular differentiation within the graft union (Albacete et al., 2015).

Phloem proteins, originating in the rootstock are also graft-transmissible and may be found throughout the scion (Golecki et al., 1998). These phloem proteins are believed to mediate the movement of RNA through the vascular system (Lucas and Lee, 2004). Such protein movement begins as soon as plasmodesmata form across the graft union (Golecki et al., 1998). Tellingly, for the proteins studied, and regardless of use as either a rootstock or scion, certain species (even across genera) were "donors", while other species were "acceptors", and certain combinations seemed to show no transmission at all, indicating that the same rootstock will likely display different (potentially compatibility-related) interactions when grafted to different scions, (Golecki et al., 1998). For this reason, it seems that while RNA may play a primary role in intercellular communication and coordination, its movement throughout the plant may be mediated by other graft-transmissible molecules.

Graft Hybridization

While the concept that the transfer and expression of short RNA sequences plays a significant role in the relative genetic compatibility of many graft pairings is well documented, the idea that such sequences could also be incorporated into the native genome of the target cells remains controversial. Such a phenomenon, known as "graft hybridization" was first proposed by Charles Darwin in his book *The Variation of Plants and Animals under Domestication*: "[T]he elements that go to the production of a new being, are not necessarily formed by the male and female organs. They are present in the cellular tissue in such a state that they can unite without the aid of the sexual organs, and thus give rise to a new bud partaking of the characters of the two parent-forms" (Darwin, 1868).

Though scientists from Russia and Eastern Europe claimed to have successfully transferred single genes from rootstock to scion via grafting prior to the 1970s, these claims were largely dismissed due to the lack of a satisfactory mechanism. However, by the mid-1970s, graft-induced heritable changes in typically mendelian traits had been observed in grafted peppers by Ohta and Chuong, (1975). Initially, this was believed to be a result of the fusion of the protoplasts of damaged cells at the graft union, since even intergeneric allopolyploids could be made in the lab by fusing the protoplasts in this way (Pandey, 1978). However, given the necrosis and callus proliferation we now know occurs as a part of the graft healing process, protoplast fusion seems highly unlikely to result from grafting. Moreover, it has been demonstrated that physical contact is unnecessary for cellular recognition and that graft healing is therefore mediated to a large extent by diffusible molecular signals, moved through the plant via plasmodesmatal streaming (Moore, 1984). So while cellular contact is important for the ultimate physical strength of the graft (Andrews and Marquez, 1993) protoplast fusion remains highly unlikely, and "graft hybridization" or asexual hybridization remains highly controversial with many dismissing such hybrids as chimeras (Albacete et al., 2015).

Mentor Grafting

It has been observed that masses of chromatin will move via the vascular system across the graft union from older rootstock tissue and into the flower buds of the younger scion tissue (Goldschmidt, 2014; Pandey, 1979). A technique known as "mentor grafting" utilizes this concept to attempt the creation of "graft hybrids". Mentor grafting involves the grafting of very young scion tissue onto mature rootstock tissue. The majority of the scions leaves are removed in order to make it completely nutritionally dependent upon the rootstock, and to encourage the influx of nucleic acids with the hope of their integration and subsequent expression in the scion's genome (Goldschmidt, 2014; Pandey, 1979). The mentor grafting technique has been well documented and has allowed for the asexual "hybridization" of varieties that would otherwise be taxonomically impossible to cross, leading to graft-induced variants whose traits were both stable and heritable (Albacete et al., 2015). Unlike a sexual hybrid however, in which half the genetic material comes from each parent, such mentor-grafted hybrids retain a substantial amount of genetic material from the original scion tissue, with relatively small segments sourced from the rootstock, typically only affecting a single trait or set of related traits. The integration of these relatively small genetic packets was verified by observing traits that had switched from homozygous dominant to homozygous recessive in a single generation, and it was noted that complimentary genes typically moved together (Pandey, 1979).

However, even with this technique, the incidence of such grafting hybrids is very low (often below 1%), though it is notable that solanaceous species seem to be more susceptible to rootstock-induced scion alteration than most (Goldschmidt, 2014; Pandey, 1979). Interestingly, the incidence of mentor grafting-induced changes was increased by more than 8x when the rootstock was inoculated with a virus prior to grafting (Pandey, 1979). Because both viruses and signaling RNAs (mediated by phloem-proteins) travel via the vascular system (Lucas et al., 2001; Xoconostle-Cazares et al., 1999), and typically target the plants meristems (Roberts et al., 1997), the two are therefore likely to be in proximity to one another, indicating that there may be a viral role in reverse-transcribing the mRNA transmitted from rootstock to scion and promoting its integration into the scions genome (Adler, 2001; Liu et al., 2010). These new traits are not expressed in the grafted or “G0” generation, and in the progeny (G1, G2, etc.), the new traits occasionally displayed “unstable chimeric mosaicism” within individual plants, though this was more common for those variants encouraged by viral inoculation (Pandey, 1979). This mosaicism, as well as information from other studies, suggests that at least some of these heritable genetic changes may not represent instances of authentic graft-induced genomic change, and may instead be a result of methylation (epigenetic gene silencing) mediated by proteins or RNA produced in the rootstock and transported into the scion via the phloem, (Albacete et al., 2015; Goldschmidt, 2014; Wu et al., 2013). Given the relatively low rate of such genetic transfer and the relatively small amount of genetic material that is transferred, even under ideal conditions, it seems that organism-wide graft-induced hybridization is unlikely to be a significant factor in the relative genetic compatibility of two grafting partners.

Horizontal Gene Transfer

Short strands of mRNA are not the only genetic material capable of moving between rootstock and scion. Large genetic packages have also been shown to cross the graft union, including entire plastid genomes (Stegemann and Bock, 2009), mitochondria (Gurdon et al., 2016), and in some cases, entire nuclear genomes (Fuentes et al., 2014; Goldschmidt, 2014). The transfer of an entire genome from rootstock to scion is known as horizontal gene transfer, and involves the formation of allopolyploid cells (Fuentes et al., 2014; Goldschmidt, 2014). Such genetic changes are typically limited to cell-to-cell exchanges in the callus area and do not alter the genome of the majority of the rootstock or scion (Fuentes et al., 2014). While it is unclear whether these allopolyploid cells play a role in graft compatibility, the tissue can form viable, fertile plants when micropropagated under laboratory conditions, even when sourced from varieties that typically form infertile hybrids when crossed sexually (Fuentes et al., 2014).

Virus-Mediated Delayed Incompatibility

It has been proposed that delayed incompatibility may frequently be due to differences in viral resistance between grafting partners (Dogra et al., 2018; Mudge, 2013; Rosskopf et al., 2013, 2014; Rowhani et al., 2017). While “virus associated delayed incompatibility” is not true incompatibility in the sense that the two grafting partners could form a strong lasting union under certain conditions, such a mechanism has been observed in grafted tomato (Rosskopf et al., 2013, 2014), as well as in grape vines, and pear (Dogra et al., 2018; Rowhani et al., 2017), walnut, citrus, and apple trees (Mudge, 2013) and presents symptoms consistent with delayed incompatibility. In this scenario, a virus-resistant (or perhaps "tolerant") variety is already hosting a virus, but showing no symptoms, and is grafted to a variety with no resistance to the virus. The graft heals normally because the grafting partners have no inherent genetic incompatibilities, but over time the virus migrates to the susceptible partner, which begins to show symptoms of incompatibility (viral infection), often including reduced growth (Mudge, 2013). For this reason, it is recommended that viral resistance compatibility (and especially resistance to Tomato Mosaic Virus) be taken into account when selecting graft pairings (Rosskopf et al., 2013, 2014).

Immune Response-Related Incompatibility

It appears that there is some degree of self and non-self graft-partner recognition, as the upregulation of genes involved in defense responses have been observed in heterografted plants to a much greater degree than in autografted plants (Albacete et al., 2015). However, while there seems to be evidence of self/non-self recognition, no specific immune-type recognition/rejection response mechanism has been identified in grafted plants (Goldschmidt, 2014), so it is still unclear what role immune-type self-recognition may play in (in)compatibility between grafting partners.

"Graft Incompatibility Toxins"

As mentioned previously, the environment can have an important effect on the interactions between grafting partners (Albacete et al., 2015). The temperature dependent graft compatibility of certain pear cultivars grafted to quince rootstock is perhaps an extreme example but suggests that not all compatibility is as genetically based as we might think. Because of such interactions, some have even proposed that graft failure may have more to do with the lethal effects of such toxins produced by one graft partner on the other partner, or the graft union itself. Additionally this might help explain why certain grafts are compatible, while their reciprocals are not (Gainza et al., 2015; Goldschmidt, 2014; Moore, 1984). Such graft failure in pear on quince grafts is believed to be the result of prunasin, a cyanogenic glucoside produced in warm climactic conditions in the quince rootstock and broken down by pear enzymes at the graft interface, releasing cyanide and causing necrosis at the graft union. (Andrews and Marquez, 1993; Gainza et al., 2015; Goldschmidt, 2014; Moore, 1984). Evidence of a similar relationship has been observed in peach on almond grafts, but only when the specific peach cultivar that is used produces higher levels of prunasin than the almond (Andrews and Marquez, 1993). However, given that the evidence for "graft incompatibility toxins" has not been observed outside of the *Prunus* genus, it is possible that this specific incompatibility mechanism may be limited to this genus.

Overcoming Graft Incompatibility

Many rootstocks are interspecific hybrids. Not only does this hybridization enhance their vigor, it also helps significantly with cross-species compatibility. For example, an interspecific F1 hybrid cross between *S. lycopersicum* cv. VF36 and *Solanum pennellii* LA0716 showed not only high vigor and disease resistance, but was also widely compatible with a variety of solanaceous species including eggplant and pepper (Albacete et al., 2015; MacDonald, 2014).

In the same way, it seems possible that the "asexual hybrid" allopolyploid cells, observed by Fuentes et al., (2014) while unlikely to give rise to new variations under regular horticultural conditions, may form a critical "interface" tissue layer between the rootstock and scion of a grafted plant and contribute to the compatibility of the grafting partners. Perhaps, in incompatible graft unions, this allopolyploid layer does not form, or is unviable due to significant differences in genome size or structure, given the tendency towards the “diploidization” of polyploids and the methylation or elimination of at least one copy of many homologous genes observed early on in the development of polyploids (Kashkush et al., 2002; Buggs et al., 2012). Such genomic instability has also been attributed to missegregation during mitosis (Fuentes et al., 2014). It is interesting that such loss or methylation included genes related to disease resistance, and cell cycle regulation (Kashkush et al., 2002), as these are important factors for the healing and compatibility of graft unions.

Cross compatible interstocks may also be used to overcome incompatibility. Such interstocks have been used successfully to overcome pear/quince incompatibility (Hartmann et al., 2013a). In a more extreme example, certain species of parasitic plants such as mistletoe and dodder have been shown to successfully interface with the vascular systems of a variety of plant species to which they are not closely related (even those from different plant families). These parasitic species may be grafted experimentally as a sort of interstock between otherwise incompatible species (Goldschmidt, 2014). Periwinkle (*Catharanthus sp.*) also seems to be able to form grafts with distantly related species in a way that other plants typically cannot (Goldschmidt, 2014). However, the availability of cross-compatible cultivars is somewhat limited (Andrews and Marquez, 1993), and further complicates both the grafting process and our understanding of the interactions between stock and scion through the addition of a third distinct genotype (Hartmann et al., 2013a). In spite of these difficulties, such interactions indicate that mechanisms independent of genetic proximity also play a role in graft compatibility, and that incompatibility related to both genetic proximity and self/non-self recognition can be overcome under certain conditions.

Research Introduction

The following studies are intended to investigate graft compatibility within the solanaceous family, and to identify both tomato and pepper graft combinations with commercial potential for high tunnel production in the Central United States. In the tomato scion variety trial, ten hybrid determinate tomato varieties were grafted to a single rootstock (‘Maxifort’) and the relationships between rootstock and scion were evaluated. The goals of this trial were as follows: 1) to determine whether rootstock effects were consistent across different scion varieties, 2) to pinpoint traits associated with and quantitatively assess compatibility, and 3) to identify grafted combinations (or nongrafted varieties) with potential for commercial, high tunnel production. Our pepper rootstock trial was intended to evaluate the compatibility of three pepper rootstocks for use with sweet bell pepper (‘Karisma’) scion and identify any graft-induced changes in the growth of the pepper plants. We also assessed the potential for intergeneric graft compatibility between the *Capsicum* and *Solanum* genera by grafting our pepper scion to two tomato varieties.

Chapter 2 - Fruit Yield and Scion Compatibility of Ten Hybrid Determinate Tomato Varieties Grafted With ‘Maxifort’ Rootstock

Summary

While tomatoes continue to be the most popular and profitable high tunnel crop, intensive cultivation and limited crop rotation can lead to a loss of soil quality and the buildup of soilborne pathogens. Many growers are adopting the use of grafted tomatoes to help address some of the challenges of high tunnel production. Previous reports surrounding grafting are typically focused on the impact of rootstock. However, little is known about the role played by scion cultivar selection or if there is a similar impact across scion cultivars regarding fruit yield when a single rootstock is utilized. In our study, ten hybrid, determinate, red slicing tomato varieties were evaluated as scions for ‘Maxifort’ rootstock and we examined fruit yield as it relates to scion compatibility. Trials were conducted in 2016 and 2017 in a three-season high tunnel in Olathe, KS. All ten of our scion varieties were found to be compatible with ‘Maxifort’. However, ‘BHN 589’, ‘Red Deuce’, ‘Skyway’, and ‘Tasti Lee’ showed significant improvements in marketable yield when grafted to ‘Maxifort’ indicating that they were “highly compatible” with the rootstock. When arranged by yield, ‘Red Deuce’ and ‘Skyway’ ranked higher among the grafted treatments than they did among the nongrafted treatments, while the opposite trend was observed for ‘Primo Red’ and ‘Scarlet Red’. This suggests that different scion cultivars display a degree of variation in their relative compatibility with ‘Maxifort’ rootstock. Our data revealed a significant inverse relationship between the nongrafted yield of the scion varieties and the percent yield benefit when grafting, suggesting that the effect of a rootstock like ‘Maxifort’ may not be synergistic, with higher performing nongrafted scion varieties benefitting less from grafting than lower performing varieties. For growers looking to utilize grafted plants, ‘Red Deuce’ and ‘BHN

589’, had the highest fruit yield of any of the treatments that we tested and ranged from to 21.4 to 23.0 lbs of marketable fruit per plant. Nongrafted ‘Primo Red’ was also a good option for high tunnel growers and provided 19.2 lbs of marketable fruit per plant. The results of this study suggest that not all scion cultivars respond to grafting with ‘Maxifort’ rootstock in the same manner and we attempted to identify ways to assess their compatibility based on crop productivity.

Introduction

Grafting has been used in greenhouse and high tunnel production systems worldwide (Kubota et al., 2008; Oda, 1999; Rivero et al., 2003). Concurrently, high tunnels have become very popular in the United States because they can extend the production season for warm season crops (Altamimi, 2010; Buller et al., 2016; Gu, 2017; Lamont, 2005; Reeve and Drost, 2012; Thompson, 2012), and allow growers to produce cool season crops through the winter in many parts of the U.S. without supplemental heat (Gu, 2017; Knewtson, 2008; Lamont, 2005). The microclimate provided by high tunnels can result in earlier production, and tomatoes (*Solanum lycopersicum*) grown in high tunnels have been shown to reach maturity as much as three weeks earlier than in the open-field (Gent, 1991; O’Connell et al., 2012). The use of high tunnels has also been shown to increase both total yield (Altamimi, 2010; Blomgren and Frisch, 2007; Jensen and Malter, 1995; Palonen et al., 2017) and crop quality (Blomgren and Frisch, 2007; Huff, 2015; Lamont, 2005; O’Connell et al., 2012).

However, the implementation of a successful high tunnel production system is not problem-free. Increased relative humidity and air temperature in combination with reduced air movement within high tunnels (Blomgren and Frisch, 2007; Gent, 1991; Kaiser and Ernst, 2012) makes an ideal environment for the spread of many fungal diseases like powdery mildew

(Mersha and Trinklein, 2015) verticillium wilt (caused by *V. dahliae*) and leaf mold (*Fulvia fulva*) (Blomgren and Frisch, 2007). The exclusion of rain and overuse of fertilizers can lead to increased soil salinity (Altamimi, 2010; Blomgren and Frisch, 2007) and blossom end rot (Kaiser and Ernst, 2012). Attempts by producers to maximize the productivity and profitability of their high tunnel structure often leads to year-round intensive cultivation (Reeve and Drost, 2012). Furthermore, many growers are reluctant to rotate high-value production space (Galinato and Miles, 2013) away from profitable solanaceous crops like tomatoes and peppers (Carey et al., 2009; Galinato and Miles, 2013; Knewtson, 2008; Lamont, 2009; Reeve and Drost, 2012). These issues have led many growers to consider the use of tomato rootstocks to improve the productivity of their high tunnels, combat soil quality or soilborne disease-related stressors (Albacete et al., 2015; Edelstein, 2004; Michel et al., 2015), and potentially increase the profitability of their operations (Rivard et al., 2010a; Rysin et al., 2015).

Many interspecific hybrids have been developed as rootstock for tomato production that have genetic disease resistance to several soilborne pathogens including root-knot nematodes, fusarium and verticillium wilt, as well as bacterial wilt (Louws et al., 2010). Grafting onto such rootstocks can be an effective disease-management tool (Bletsos and Olympios, 2008; Blomgren and Frisch, 2007; Edelstein, 2004; Fernández-García et al., 2004; Lee, 1994; ‘Tomato Grafting’, 2015). Grafting can also improve plant tolerance of harsh growing conditions when certain rootstocks are utilized, including soil temperature (Bletsos and Olympios, 2008; Edelstein, 2004), high soil salinity (Haberal et al., 2016), and flooding (Bahadur et al., 2015; Kubota et al., 2008; Petran, 2013; Schreinemachers and Afari-Sefa, 2012; ‘Tomato Grafting’, 2015; Wu et al., 2012). Grafting with specific rootstocks has also been shown to improve overall plant health and vigor (Albacete et al., 2015; Djidonou et al., 2013; Haberal et al., 2016; Lee, 1994; Masterson et al., 2016b), leading to improvements in both yield (Bletsos and Olympios, 2008; Davis et al., 2008b; Djidonou et al., 2013; Edelstein, 2004) and fruit quality (Davis et al., 2008b; Frey et al., 2017; Haberal et al., 2016). Grafted plants may have larger root systems (Djidonou et al., 2013; Lee, 1994), be more efficient at moving water and nutrients (Djidonou et al., 2013; Haberal et al., 2016; Lee, 1994), and have greater vegetative production (Djidonou et al., 2013; Haberal et al., 2016).

‘Maxifort’ is a well-known, vigorous, and widely used F1 interspecific hybrid tomato rootstock (*S. lycopersicum* x *S. Habrochaites*) (McAvoy, 2005; Bletsos and Olympios, 2008; Buller et al., 2013; Masterson et al., 2016b ; Hu and Kleinhenz, 2015; Hu, 2016; Meyer, 2016), and is considered by some to be the “standard” rootstock for grafting trials in the US (AGIS, 2015). ‘Maxifort’ and the very similar ‘Multifort’ are both available from De Ruiter/Monsanto (Bergschenhoek, The Netherlands) and are good examples of vigorous and resistant tomato rootstocks. ‘Maxifort’ has been reported to reduce the symptoms of and/or confer resistance to soilborne plant pathogens like fusarium wilt (Rivard and Louws, 2008), root-knot nematodes and southern blight (Rivard et al., 2010b), and verticillium wilt (Louws et al., 2010). ‘Maxifort’ and ‘Multifort’ rootstocks have been tested with numerous scion varieties including ‘BHN 589’ Masterson et al., 2016b ; Meyer, 2016), ‘Brandywine’, ‘Flamme’ (Barrett et al., 2012) ‘Celebrity’ (Hu, 2016), Cherokee Purple (Buller et al., 2013; Masterson et al., 2016b ; Rivard et al., 2010b), ‘Florida 47’ (Djidonou et al., 2013), German Johnson (Rivard and Louws, 2008), Garden Gem, and Tribute (Frey et al., 2017). Both ‘Maxifort’ and ‘Multifort’ have been shown to enhance plant biomass and leaf area and improved yield in growing systems with little to no disease pressure or abiotic stress as measured by both fruit number and weight (Barrett et al., 2012; Djidonou et al., 2013; Frey et al., 2017; Masterson et al., 2016b; Rivard and Louws, 2008) and plants grafted to ‘Multifort’ have also demonstrated increased efficiency of N fertilizer and water uptake (Djidonou et al., 2013). Economic studies have also shown that the implementation of ‘Multifort’ can improve crop profitability (Djidonou et al., 2013).

As a result of the benefits that rootstocks can provide, grafted plants often excel in covered production systems (like high tunnels) as compared to nongrafted plants (Frey et al., 2017; Oda, 1999). Not only are they able to address issues that are prevalent in high tunnels like soil salinity and soilborne diseases (Buller et al., 2016; Giles, 2011; Rodriquez and Bosland, 2010; Oda, 1999), but they may also be better-equipped to take advantage of the improvements offered by the high tunnel growing environment. Although grafting tomatoes for high tunnel production has many potential advantages, one possible disadvantage is the potential for graft incompatibility and the physiological disorders associated with it (Edelstein, 2004).

Graft compatibility is presumed to have a strong genetic component (Dogra et al., 2018) and may involve the movement and expression of genetic material between rootstock and scion as demonstrated in sunflower (*Helianthus*), tobacco (*Nicotiana tabacum*) (Albacete et al., 2015; Lucas et al., 2001; Zhang et al., 2014), tomato (Lucas et al., 2001; Wu et al., 2013; Velez-Ramirez et al., 2014), Eggplant (*S. melongena*) (Wu et al., 2013), pepper (*Capsicum Annum*) (Bletsos and Olympios, 2008) cucumber (*Cucumis sativus*), pumpkin (*Cucurbita maxima*) (Ruiz-Medrano et al., 1999), and Arabidopsis (*A. thaliana*) (Zhang et al., 2014). However, compatibility is still complex, highly variable, and difficult to predict with precision (Andrews and Marquez, 1993; Venema et al., 2011). While there seems to be no specific definition for graft compatibility, Goldschmidt (2014) outlined three basic characteristics of compatible grafting partners: 1) The establishment of a successful graft union 2) The extended survival of the composite plant, and 3) The proper functioning of the composite, grafted plant. Edelstein

(2004) and Gainza et al.,(2015) elaborated on these concepts when describing basic symptoms of incompatibility. These symptoms included a failure of the rootstock and scion to unite, a lack of health, graft breakage, and the premature death of the plant or failure of the graft union, and the inability for the grafting partners to form a strong, lasting, and functional union, Hartmann et al., (2014) summarizes the concept, “The ability of two different plants, grafted together, to produce a successful union and to develop satisfactorily into one composite or compound plant” (p. 459).

Immediate graft incompatibility, characterized by the rapid death of the scion tissue, is attributed to the failure of the vascular tissue to heal and function properly, and typically becomes apparent shortly after grafting (Mudge, 2013). Other times, incompatibility may be delayed, though it is still associated with a set of recognizable symptoms, including growth inhibition, high leaf chlorophyll content, undersized fruit, wilting, high rates of mortality (Kawaguchi et al., 2008; Oda et al., 2005), and scion over or under-growth, (Gainza et al., 2015; Goldschmidt, 2014; Kawaguchi et al., 2008; Oda et al., 2005).

A rootstock / scion combination may form a strong, lasting, and functional graft union without displaying overt symptoms of incompatibility, however, it may still be considered “moderately incompatible”. Moderate graft incompatibility is observed in interspecific eggplant/tomato grafts (Goldschmidt, 2014). This incompatibility is characterized by reduced plant size, a decrease in heterograft water potential (Oda, et. al., 2005), and reductions in fruit yield and quality. These findings were attributed to differences in the nutrient requirements between eggplant and tomato (Kawaguchi et al., 2008; Oda, et. al., 2005). Since the vascular connections still appear to be relatively normal, such moderate incompatibility may still be tolerable or even desirable under certain growing conditions. For example, severely waterlogged soils create a significant barrier for the production of tomatoes unless they are grafted onto eggplant rootstocks (Bahadur et al., 2015).

Incompatibility is typically identified based merely on observable symptoms including low percent graft “take”, breakage or anatomical abnormalities at the graft union, asynchronous stock/scion growth, and vascular or cambial discontinuity (Mudge, 2013). While potential graft pairings are frequently described as either compatible or incompatible based on the presence or absence of such symptoms, some have attempted to quantify compatibility based on grafting success rates (Yetişir et al., 2007). However, descriptions of moderate incompatibility by Oda et al.,(2005), Kawaguchi et al.,(2008), and Goldschmidt, (2014) suggest quantifiable long-term yield and morphological attributes that may be used to compare the relative compatibility of multiple graft pairings.

It is important to differentiate between high graft compatibility and environmental tolerance, since many grafting studies report significant environmental interactions (Soltan et al., 2017). Because the act of grafting creates a plant with a composite genotype, a simple Genotype x Environment approach is insufficient, and while more complex; a Rootstock x Scion x Environment approach is more useful (Albacete et al., 2015; Leal-Fernández et al., 2013). Attempting to control for the effect of the environment by choosing highly productive environments with few biotic or abiotic stressors encourages the genotypes being tested to express their full genetic potential, which allows for a more accurate genotype-to-genotype assessment with more easily discernable differences in relative compatibility (Hayward et al., 1993).

There have been a number of studies with grafted tomatoes, involving a diverse assortment of scion cultivars and/or nongrafted controls. Such scion cultivars include hybrids, heirloom varieties, and on occasion unreleased lines (Velez-Ramirez et al., 2017; Haberal et al., 2016). Hybrid tomato cultivars that have been utilized as scion include determinate tomatoes: ‘BHN 589’ (Masterson et al., 2016b; Meyer et al., 2017), ‘Florida 47’ (Djidonou et al., 2013) (Sen et al., 2018), and ‘Tribute’ (Frey et al., 2017; Zhao et al., 2014), Semi-Determinate varieties: ‘Arka Rakshak’ and ‘Arka Samrat’ (Bahadur et al., 2015), ‘Celebrity’ (Hu and Kleinhenz, 2015; Petran, 2013), and ‘Garden Gem’ (Frey et al., 2017), and indeterminates: ‘Better Boy’ (Hu and Kleinhenz, 2015), ‘Boludo’ (Asins et al., 2015), and ‘Momotaro’ (Oda et al., 2005). Heirloom varieties are also frequently grafted due to the potential for added disease resistance (Rivard and Louws, 2006). These are typically indeterminate varieties such as ‘Brandywine’ (Barrett et al., 2012; Hu and Kleinhenz, 2015), ‘Cherokee Purple’ (Hu and Kleinhenz, 2015; Rivard et al., 2010b; Masterson et al., 2016b), ‘German Johnson’ (Rivard et al., 2010b; Rivard and Louws, 2008) and ‘San Marzano 2’ (Hu and Kleinhenz, 2015), but may also include determinate varieties like ‘Heinz-2274’ (Haberal et al., 2016).

‘BHN 589’ is often grafted to and marketed with ‘Maxifort’ rootstock as the two varieties are reported to be highly compatible (Johnny’s Selected Seeds, 2018a). ‘BHN 589’ is known to be highly productive in a high tunnel environment, both on its own (Maynard and Bluhm, 2018; Oxley and Rivard, 2015, 2016; Rivard et al., 2014b) and when grafted to ‘Maxifort’ (Masterson et al., 2016b).

Although there have been numerous reports in the literature recently that involve grafted tomato plants, these trials are typically designed to compare rootstocks or to analyze the effectiveness of specific growing practices or production systems that include grafted plants and often vary in design from site-to-site. While a wide variety of tomato cultivars have been evaluated as scions in grafting trials, most such trials focus on one scion cultivar, and there are few, if any, reports where more than two scion varieties are utilized in a single trial. This is probably due to the space requirements of a grafting trial testing multiple scion cultivars in addition to the regional and/or market distribution of tomato variety preference by growers. In spite of the number of trials that have been completed, this means that it is still unclear whether the effects of grafting with a particular rootstock are consistent across different scion cultivars or for the same cultivars grown under different environmental conditions (Davis et al., 2008a; Albacete et al., 2015). Therefore, our objectives were to: 1) determine whether the effects of 'Maxifort' rootstock on yield and plant biomass are consistent across ten scion cultivars under little to no disease pressure; 2) assess the quantitative compatibility of 'Maxifort' with ten scion varieties as it relates to plant productivity and identify any plant growth traits that are consistent with high compatibility; and 3) identify nongrafted or grafted rootstock and scion combinations that show commercial potential for high tunnel production systems due to their fruit yield and productivity.

Materials and Methods

Trials were conducted in 2016 and 2017 in a three season, multi-bay high tunnel (Haygrove, Ledbury, UK), at the Kansas State University Olathe Horticulture Research and Extension Center (OHREC), located in Johnson County, Kansas (lat. 38.894409N, long. 94.995437 W). The soil type at this location is a chase silt loam. Each bay measured 24 x 200 ft and a different bay was used each year. In both years, a randomized complete block design was utilized with four complete replications (or blocks). Each trial was located centrally within the high tunnel bay. Replications were arranged side by side and each consisted of a single 180' long row divided into twenty plots. Each plot contained five grafted or nongrafted plants of a single scion cultivar and was randomly assigned a location within its respective replication. The list of scions being tested was based on a list of varieties that performed well in previous (nongrafted) tomato variety trials in high tunnels at OHREC (Oxley and Rivard, 2015, 2016; Rivard et al., 2014b). In both years, ‘Maxifort’ was utilized as the rootstock and ten scion varieties were investigated: ‘BHN 589’, ‘Fletcher’, ‘Primo Red’, ‘Red Deuce’, ‘Red Morning’, ‘Richmond’, ‘Scarlet Red’, ‘Skyway’, ‘Summerpick’, and ‘Tasti Lee’ (Table 2.1). Every replication in both years included all ten scions grafted to ‘Maxifort’, as well as a nongrafted control of each variety as a standard comparison.

Our goal was to evaluate these ten scions for compatibility with ‘Maxifort’ rootstock and to observe any associated changes in yield or biomass production. Therefore, trials were intentionally located in areas that had no history of soilborne disease problems, and regular plant observations found no evidence of any major soilborne or foliar disease in either year.

Transplant Production and Grafting

All grafted and nongrafted transplants were produced at the Kansas State University Olathe Horticulture Research and Extension Center (Olathe, KS). Grafting was performed using the tube-grafting technique, also known as splice grafting or Japanese top-grafting (MacDonald, 2014; (Masterson et al., 2016a; McAvoy, 2005; Meyer et al., 2017; Oda, 1999; Rivard and Louws, 2006). Prior to grafting, approximately 80-90% of the leaf area of the scion was removed and care was taken not to damage the apical meristem as outlined by Masterson et al., (2016a) and Meyer et al., (2017). Rootstock and scion seedlings were grown in soilless potting medium and were grafted when they were approximately three and a half to four weeks old (at the two to four true leaf stage). Grafted seedlings were held together for the duration of the healing process with a silicon clip (Hydro-Gardens, Colorado Springs, CO). All grafted seedlings were subsequently placed inside a healing chamber with a polyethylene film covering, 2-3 layers of

55% shade cloth, and a supplemental cool-mist humidifier. Established healing chamber management protocols developed for tomatoes were followed (Rivard and Louws, 2010; Rivard et al., 2010a). Following graft union formation, all grafted seedlings were removed from the healing chamber and grown in the greenhouse for at least 14 d to allow for re-acclimation to greenhouse conditions before transplanting into the high tunnel.

Growing Methods

Cultural methods were consistent with commercial high tunnel tomato production. In-row plant spacing was 18 inches with one empty space left between plots. Rows were 5 ft apart (center-to-center), and plants were grown in a stake and weave trellis system. A plasticulture, raised-bed growing system was used, and weeds were suppressed via woven fabric mulch between rows and polyethylene mulch (1.25 mil) over the beds. Water was applied throughout the growing season by drip irrigation. Integrated pest management practices typical for high tunnel tomato production were used, and weekly or biweekly applications of Bt (*Bacillus thuringiensis*) based organic pesticide were based on pest pressure.

2016 Trial

Soil tests indicated that the soil pH was 7.2 within the bay that was utilized in 2016. Pre-plant fertilizer (calcium nitrate; 15.5-0-0) was applied at 50 lbs N/acre. Four applications of water-soluble nitrogen fertilizer were applied on a scheduled basis via fertigation at a rate of 10 lbs N/acre on 29 April, 1 and 30 June, and 1 Aug. The first and third applications used potassium nitrate (13-0-46), while the second and fourth applications utilized calcium nitrate (15.5-0-0). The 2016 trial was planted on 18 April, 2016, and harvesting occurred on 28 June, 5, 11, 14, 19, and 25 July, 1, 5, 9, 12, 22, and 29 August, and 6, 13, 19, and 27 September.

2017 Trial

In the 2017 trial, soil tests indicated that the pH in this bay was 6.8. Custom-blended granular fertilizer (31-16-16) was applied at a rate of 50 lbs N/acre, prior to planting. Similar to 2016, water-soluble fertilizer was also applied via fertigation at a rate of 10 lbs N/acre. However, fertilizer use was based the results of weekly tissue analysis with a LAQUAtwin handheld nitrate meter (Horiba Instruments Inc.; Irvine, CA), and fertilizer was applied only when leaf tissue % nitrogen was below the recommended levels of 3.5-4.0 when fruiting or 4.0-5.0 prior to fruiting (Grubinger, 2010; NCDAandCS, 2011). In 2017, fertigation was only conducted once, and potassium nitrate was applied on 4 Aug. The trial was planted on 2 May, 2017, and harvesting occurred on 3, 10, 16, 24, and 31 July, 7, 14, 19, and 28 August, 5, 11, 18, and 24 September, and 2 October.

Data Collection

There were 91 days between the first and last harvest in both years. All tomato fruit were harvested at the “breaker” stage (Cantwell, 2013). Total fruit yield was graded into marketable and unmarketable fruit based on the presence of fruit diseases, blossom end rot, and/or pest damage. For each plot, fruit weight and number were recorded for both marketable and unmarketable fruit. On the day of the final harvest of each growing season, all undamaged green fruit larger than 1.5 inches in diameter were harvested and included in the marketable yield. In 2017, two centrally-located plants were destructively sampled from each plot after the last harvest. Above-ground biomass samples were taken by cutting the stem at approximately one centimeter above the soil. Below-ground biomass samples were collected using a shovel to collect as much of the root system as possible. Roots were washed prior to drying and both above and below-ground biomass samples were dried for at least 72 hours at 160°F (71.1°C) using a

Grieve Industrial Shelf Batch Oven model SC-400 (The Grieve Corporation, Round Lake, Illinois). Per-plant biomass data for each plot was calculated as the mean of the two plants that were sampled

Data Analysis

All raw yield data were first converted to a per-plant basis and were analyzed in SAS Studio: University Edition (version 9.4; SAS Institute Inc. Cary, NC). An ANOVA found significant year*treatment interactions that occurred when the data from the two trials were combined. It is unclear what the primary factor in this difference was. Though the high tunnel allowed for some control over the study's microclimate, differences in weather and N fertilizer application from year-to-year may have played a role in this interaction. To control for this difference, data that was available for both years was standardized for the independent variable "year" using the z-score standardization procedure (z-score = $(x - \mu) / \sigma$). This procedure assumes each data set is normally distributed (RGalleon, 2018) and then standardizes it around a mean of "0" and a standard deviation of "1"(Field, 2005; SPSS, 2016; Bhalla, 2017) without changing the distribution (SPSS, 2016). This allows for the comparison of certain independent variables (graft status and scion variety) across multivariate data sets, which have significant differences in mean, range and standard deviation (Field, 2005; SPSS, 2016; Bhalla, 2017; Shaw and Brown, 2017; Ling, 2018) without affecting any relationships within those data sets.

A three-way repeated-measures factorial ANOVA was used to check for statistical interactions and to compare main and simple effects (Table 2.2). The three factors that were tested included year, graft status (grafted vs. nongrafted), and scion variety. Where significant effects were identified, a mean separation test was carried out using Tukey's Honest Significant Difference Test ($\alpha = 0.05$). The least squares mean values for the transformed data were also back-transformed after testing (Manikandan, 2010) for presentation of the data in the tables. Simple arithmetic (raw) means were calculated for each of the dependent variables, although this value is equal to any calculated least squares means since all varieties tested had the same sample size (Deng, 2009). Even after z-score standardization, factorial ANOVAs found significant interactions with year for both marketable and total fruit number and size (Table 2.2). Therefore, factorial analysis for these dependent variables was carried out independently for each year for these parameters.

No data was found to deviate significantly from variance homogeneity when tested using Levene's Test for Homogeneity; additionally, goodness-of-fit tests for the normal distribution concluded that most data had an approximately normal distribution. Therefore, for marketable and total yield (lbs) (Table 2.3), marketable and total fruit number (Table 2.3), marketable and total fruit size (lbs/fruit) (Table 2.3), and graft-induced changes in marketable yield (lbs and percent) (Table 2.4), a standard, one-way Analysis of Variance (ANOVA) with a Tukey's Honest Significant Difference (HSD) post-hoc test was used to check for statistically significant differences ($\alpha = 0.05$), both between cultivars and between grafted and nongrafted versions of the same cultivar.

In an effort to address the second objective of this study (the comparison of relative compatibility), Table 2.4 was created to show the changes in actual and normalized (percent)) marketable yield data between grafted and nongrafted plants for each scion variety. Change in weight includes the difference between the (per plant) grafted and non-grafted treatment within each replication. Similarly, normalized yield data shows the % change in marketable yield of the grafted plants as compared to the yield of the nongrafted plants (% change in yield = (grafted plant yield / nongrafted plant yield) - 1).

Table 2.5 was also created to help address objective two. The rankings in Table 2.5 were based simply on the mean values for marketable and total yield by weight, and the statistical significance of yield differences was not taken into account. The assumption being that if all varieties show an equal degree of benefit to grafting on ‘Maxifort’ and therefore similar levels of compatibility, the rankings of the nongrafted plants should be approximately the same as the rankings of the grafted plants.

Biomass data (Table 2.6) was available for only the 2017 season and was therefore not transformed with the z-score standardization procedure. However, both above and below-ground biomass data failed goodness-of-fit tests for the normal distribution. Therefore, biomass data was transformed logarithmically prior to analysis. The transformed data was found to be normally distributed, and standard, one-way ANOVAs with Tukey’s HSD post-hoc tests were used to evaluate the data.

ANOVAs with Tukey’s HSD post-hoc tests were also used to compare marketable and total fruit number for each year independently as a function of graft status, as well as above and below-ground biomass across all scion varieties as a function of graft status (grafted vs nongrafted).

Correlation analysis (Table 2.7) were also used to analyze the relationships between above and below-ground biomass and marketable yield (measured by both fruit number and weight), as well as the relationship between nongrafted yield and the percent change in yield due to grafting. Pearson product-moment correlation was used to determine the strength and direction of the linear correlation for any relationships found. In some cases, linear regression analysis was used to further examine the relationships.

Results

Main and Combined Effects

The main effects of graft status and scion were found to be significant for all aspects of both marketable and total fruit yield, and were the strongest effects observed overall (Table 2.2). However, the year*graft status as well as the year*scion interactions were significant for (both marketable and total) fruit size as well as number (Table 2.2). Therefore, each year's data are presented separately in Table 2.3 for these parameters but are combined for marketable and total fruit yield (lbs/plant). While the graft status*scion interaction was never statistically significant (Table 2.2), the simple effects are shown in Table 2.3 to inform the reader of trends that occurred between grafted and nongrafted plants of all ten varieties.

Impact of Scion Variety and Grafting on Fruit Yield

Significant increases (40.1% to 52.8%) in marketable yield (lbs/plant) over corresponding nongrafted plants were observed for four scion cultivars ('BHN 589', 'Red Deuce', 'Skyway', and 'Tasti Lee') when 'Maxifort' rootstock was utilized ($P<0.05$; Table 2.3). No such significant grafting effects were observed for total yield (lbs/plant) (Table 2.3). Numerical, though not always significant, increases in both marketable and total fruit yield (lbs/plant) were observed for all varieties and no varieties were penalized by grafting with 'Maxifort' rootstock (Table 2.3).

The factorial analyses for total and marketable fruit number were carried out separately for each year (Table 2.3), and comparisons of nongrafted scion varieties to their grafted counterparts found no significant differences between nongrafted plants and their grafted counterparts for the number of total or marketable fruit produced per plant for either year (Table 2.3). While grafted plants produced significantly more total and marketable fruit ($P < 0.001$ for both) than nongrafted plants in 2017, no such benefit was apparent in 2016 for numbers of either total (*P* = 0.5670) or marketable (*P* = 0.6339) fruit produced (data not shown). In both years, there were significant differences between the highest and lowest-producing varieties regarding marketable and total fruit number (Table 2.3).

Impact of Scion Variety and Grafting on Fruit Size

Because significant year x graft status interactions were observed for both marketable and total fruit size, the factorial analysis for these variables was carried out separately for each year (Table 2.2). Numerical, though not always significant, increases in both marketable and total fruit size were observed for all varieties in both years and no varieties were penalized by grafting with ‘Maxifort’ rootstock (Table 2.3). In 2016 grafting significantly improved the marketable fruit size of ‘Primo Red’, ‘Red Deuce’, and ‘Richmond’ by 31.7 - 35.9% (Table 2.3) as compared to their nongrafted comparisons. During the 2017 trial, grafting to ‘Maxifort’ rootstock significantly improved the marketable fruit size of ‘Richmond’, ‘Skyway’, and ‘Summerpick’ by 27.7 - 33.3% (Table 2.3) over their nongrafted controls.

Observable trends in total fruit size were similar to trends for marketable fruit size. While the increases in total fruit size were significant for all but three varieties (‘Fletcher’, ‘Summerpick’, and ‘Tasti Lee’) in 2016, significant differences in total fruit size between nongrafted plants and their grafted comparisons were observed for only three varieties (‘Richmond’, ‘Skyway’, and ‘Summerpick’) in 2017 (Table 2.3).

Graft-Induced Changes in Marketable Yield

All grafted scion varieties showed at least numerical improvements in marketable yield over their nongrafted counterparts. Table 2.4 displays these changes in actual and normalized marketable yield data between grafted and nongrafted plants for each scion variety. There were no statistically significant differences in graft-induced per-plant marketable yield change (by weight) for a 95% confidence interval between any two scion cultivars. However, significant differences in percent changes in total yield were found between ‘Tasti Lee’ which displayed the highest percent improvement, and ‘Primo Red’ which improved the least of any variety, and the marketable yield of nongrafted plants was found to be inversely correlated with the percent change in marketable yield due to grafting (Pearson Correlation Coefficient = -0.46778, *P* <0.0001).

Impact of Grafting and Scion Variety on Yield Ranking

Table 2.5 shows the rankings of grafted and nongrafted plants based on marketable and total fruit yield (lbs/plant) to compare the effects of grafting across varieties and determine what nongrafted or grafted rootstock and scion combinations show potential for high tunnel production. ‘Primo Red’ had the highest productivity amongst the nongrafted plants for both total and marketable yield amongst the nongrafted treatments. However, amongst the grafted treatments, ‘Primo Red’ had only the fourth highest marketable and total yield (Table 2.5). Similarly, nongrafted ‘Scarlet Red’ had the fifth highest marketable and total yield compared to other nongrafted varieties but fell to ninth amongst the grafted plants for both parameters. Conversely, ‘Red Deuce’ and ‘Skyway’ followed an opposite trend. ‘Red Deuce was ranked third amongst nongrafted treatments but ranked first amongst the plants grafted onto ‘Maxifort’ for marketable and total yield (Table 2.5). When the plants were grafted, Skyway’ also moved up in the rankings; from ten to seven for marketable yield, and from seven to five for total yield.

Impact of Grafting and Scion Variety on Plant Biomass, Relationship to Yield

Above and below-ground biomass were significantly affected by both grafting and scion variety (Table 2.2). For all scion varieties, both above and below-ground biomass were increased significantly (by an average of 57.2% and 36.6% respectively) when plants were grafted to

‘Maxifort’ rootstock. Though there were no interaction effects, ANOVAs comparing above and below-ground biomass for different scions found that ‘BHN 589’, ‘Richmond’, and ‘Summerpick’ showed significant increases in above-ground biomass (*P* < 0.05; Table 2.6), and all scion varieties showed numerical improvements in above-ground biomass when grafted to ‘Maxifort’ rootstock. While some significant scion-to-scion differences in below-ground biomass were present, no significant improvements for individual scion varieties were observed when they were grafted (Table 2.6).

Above and below-ground biomass were each found to have a significant positive correlation with marketable as well as total yield as measured by both fruit number and weight (Table 2.7). Above-ground biomass was slightly more strongly correlated with marketable and total yield (lbs of fruit per plant) than below-ground biomass. For above-ground biomass, the strongest overall correlations were observed for yield measured by weight, whereas for below-ground biomass, correlation strength was similar for yield measured by both fruit weight and number. Interestingly, only above-ground biomass showed a significant relationship with fruit size (Table 2.7). Above and below-ground biomass were found to be strongly positively correlated with a Pearson Correlation Coefficient of 0.76903, and a *P* value of <0.0001. A follow-up linear regression analysis, found that below-ground biomass was predictive of about 59% ($r^2 = 0.5914$) of the variance observed in above-ground biomass (data not shown).

Discussion

Rootstock and Scion Relationship Dynamics

Because no varieties showed significant (or even numerical) decreases in yield when grafted, all the scions that were tested in this study appear to be compatible with ‘Maxifort’ rootstock. More specifically, ‘BHN 589’, ‘Red Deuce’, ‘Skyway’, and ‘Tasti Lee’ produced significantly more marketable fruit when grafted with ‘Maxifort’ and may be considered highly compatible varieties. This is consistent with previous studies that report improved yields for plants grafted with ‘Maxifort’ (Masterson et al., 2016b; Rivard and Louws, 2008) and the very similar ‘Multifort’ (Barrett et al., 2012; Djidonou et al., 2013; Frey et al., 2017) rootstocks. A similar trend of increased biomass production for plants grafted to ‘Maxifort’ rootstock was also evident, and was consistent with previous reports by Barrett et al.,(2012) and Masterson et al., (2016b). We also observed an increase in fruit number for plants grafted with ‘Maxifort’ rootstock which was consistent with what was reported by Djidonou et al.,(2013), Masterson et al., (2016b), and Rivard and Louws (2008). Our results also suggest that the trial-to-trial and scion-to-scion consistency of improvements in yield attributed to the use of ‘Maxifort’ rootstock reported by Barrett et al.,(2012), Djidonou et al.,(2013), Frey et al.,(2017) Masterson et al., (2016b), and Rivard and Louws (2008) may be related to the production of larger fruit. We observed a significant overall increase in fruit size for plants grafted onto ‘Maxifort’ rootstock, which was also reported by Masterson et al., (2016b) and Meyer (2016). While most data showed similar year-to-year trends, the year-to-year improvement in fruit size by variety was highly inconsistent, suggesting that both grafting, and the environment had a strong impact on fruit size.

Based on our data, it is not clear whether the ten scion varieties we tested are responding consistently when grafted with ‘Maxifort’ rootstock. To our knowledge, a preliminary report by AGIS, (2015) is currently the only other publication comparing the performance of ‘Maxifort’ rootstock across multiple scions, and there is some scion-to-scion variability. However, the AGIS, (2015) report is based on a meta-analysis with limited control over environmental variables, making it difficult to determine if the scions are differentially-impacted by grafting with ‘Maxifort’ rootstock or if environmental or cultural management practices at different trial sites are playing a more important role.

In our trial, four varieties (‘BHN 589’, ‘Red Deuce’, ‘Skyway’, and ‘Tasti Lee’) had significantly higher marketable fruit yield when they were grafted onto ‘Maxifort’ rootstock whereas the other six did not. Furthermore, dramatic, if not statistically significant, numerical differences were observed between cultivars in terms of the numerical change of marketable fruit yield and there was a significant effect on the normalized (percent) change. Fruit number and fruit size were also impacted differentially by grafting with various scion varieties. These parameters may be less meaningful for understanding the economic impacts of grafting, as most tomatoes are marketed by weight. However, they may still provide useful information to growers that are looking to access markets where specific fruit sizes are be preferred. The absence of a significant graft status*scion variety effect indicates that scions responded similarly to grafting with ‘Maxifort’ rootstock, However, it is worth noting that fairly strong differences in the numerical change of marketable yield due to grafting seem to exist between the scion cultivars tested in this study (Table 2.4).

The greatest graft-induced percent and total improvements in marketable yield (by weight) occurred for the lowest yielding nongrafted varieties like ‘Skyway’ and ‘Tasti Lee’, which improved by 6.0 and 7.0 lbs respectively (56.4% and 59.9%) when grafted. The highest yielding nongrafted variety, ‘Primo Red’, improved by only 1.5 lbs (9.2%) when grafted onto ‘Maxifort’ and the difference in percent change between ‘Primo Red’ and ‘Tasti Lee’ was statistically significant (Table 2.4). Furthermore, when ranked by marketable yield (by weight), ‘Tasti Lee’ was intermediate both as a nongrafted plant among other nongrafted plants, and when grafted and among grafted plants. However, the ranking of ‘Primo Red’ drops from the very top of the nongrafted plants to only just above 'Tasti Lee' when compared to other grafted plants (Table 2.5). These observations lend support to the significant inverse relationship we observed between nongrafted yield and the percent change in yield due to grafting. The general trend is therefore toward a greater grafting benefit for otherwise lower-performing varieties. So while the general interactions between 'Maxifort' and a variety of scion cultivars occur in a consistent (positive) direction, the magnitude of those interactions appears to vary quite a bit from scion to scion as noted in the report published by AGIS (2015). Therefore, the effect of the rootstock on the composite plant may not be synergistic, as higher performing scion varieties have less to gain from the replacement of their (presumably higher performing) native root system with a high performing rootstock.

Although the graft-status*scion interaction was not a significant effect for any measurement (Table 2.2), when the data was divided by both graft status and scion variety, there were clear cultivars-to-cultivar differences in grafting response. It is unclear why our statistics do not agree in this regard, although sample size and study design may play a role. In this study, we used a randomized complete block design with only four replications and did not include a split-plot arrangement. Future work may benefit from a larger number of replications (perhaps with fewer scions) and a split-plot design that utilizes scion as a main plot and graft-status as the sub-plot.

Determining Rootstock and Scion Compatibility

When a solanaceous plant is grafted with a rootstock, a variety of general outcomes may occur: 1) The graft union may fail to form, and one or both of the graft partners may die, such is the case with acute incompatibility (Hartmann et al., 2013b) ; 2) The graft union may display "delayed incompatibility" and the graft union may appear to heal and function for some time but with maturity, the graft union weakens, and the grafted plant may die or show a severe reduction in productivity and plant growth (Gainza et al., 2015; Goldschmidt, 2014; Hartmann et al., 2013b; Kawaguchi et al., 2008; Oda et al., 2005); 3) The graft pairing is "moderately incompatible" and while the graft union is generally functional, the grafted plant displays a somewhat reduced level of productivity or plant growth, but does not display other, overt symptoms of incompatibility (Goldschmidt, 2014; Kawaguchi et al., 2008; Oda, et al., 2005); 4) In some graft pairings, the graft union may be compatible and function well, though the grafted plant shows no substantial differences in growth or yield as compared to nongrafted plants (Leal-Fernández et al., 2013) (See Ch 3, pg 104 & 108 regarding 'Meeting' pepper rootstock) 5) the graft pairing may instead be "highly compatible" and the grafted plant may be more productive and function at a higher level than nongrafted plants, even in situations with little disease pressure or abiotic stress (Barrett et al., 2012; Djidonou et al., 2013; Frey et al., 2017; Masterson et al., 2016b; Rivard and Louws, 2008).

Descriptions of moderate graft incompatibility by Oda et al.,(2005), Kawaguchi et al.,(2008), and Goldschmidt, (2014) suggest that quantifiable attributes like yield and plant size may be used to compare the relative compatibility of multiple graft pairings. In this study, we normalized the benefit of grafting based on the yield of the nongrafted plant. We were therefore able to quantitatively assess the benefits of 'Maxifort' when grafted with ten different scions. In our study, we did not identify any scions that were acutely incompatible with 'Maxifort', nor did any scions demonstrate a late-season decline in health or overall decreased productivity (symptoms of delayed or moderate incompatibility) when grafted onto 'Maxifort' rootstock.

The varieties tested in our study fall in three general categories regarding compatibility, though the boundaries of these categories are not well defined, and more work is needed to clarify them. ‘Primo Red’, ‘Scarlet Red’, and ‘Red Morning’ had marketable fruit yield increases that ranged from 9.2% to 25.1%. Because these varieties showed no statistical differences between grafted and nongrafted plants (no productive losses due to grafting), they could be considered “compatible” with ‘Maxifort’ rootstock. Conversely, we found that grafting with ‘Maxifort’ rootstock had a significant positive effect on the marketable yields of ‘BHN 589’, ‘Red Deuce’, ‘Skyway’, and ‘Tasti Lee’, in a high tunnel setting, which ranged from 41.0% to 59.5%. These “highly compatible” scion varieties consistently provided more than 40% increases in fruit yield. The other varieties that were evaluated could be considered moderately compatible and exhibited graft-induced fruit yield increases ranging from 25% to 40%.

Plant growth can also be utilized to assess compatibility and both above and below-ground biomass was correlated with fruit weight and number. A large improvement in yield (lbs/plant) was seen with ‘BHN 589’ and this variety also showed significant improvements in above-ground biomass when grafted to ‘Maxifort’ rootstock. Similar results have been seen with ‘BHN 589’ in high tunnel studies in the past (Masterson et al., 2016b; Meyer, 2016). This trend of increased biomass production when ‘Maxifort’ rootstock was utilized (while not significant for all varieties individually) was consistent and statistically significant when the data from all scion cultivars was combined. This is consistent with ‘Maxifort’s reputation in the industry as a vigorous and/or vegetative rootstock (Barrett et al., 2012; Djidonou et al., 2013; Frey et al., 2017; Masterson et al., 2016b; Rivard and Louws, 2008) and is in agreement with the high degree of compatibility with ‘BHN 589’ described by Masterson et al., (2016b**)**, Maynard and Bluhm (2018), and Meyer (2016).

By ranking the performance of each scion variety amongst a population, we may gain greater insight into the potential impact of rootstock to provide a benefit to the scion and therefore identify scion and rootstock combinations that are highly compatible or display quantitative compatibility. In this study, we ranked each scion within the population of nongrafted and grafted treatments. For six of the scion varieties tested (‘BHN 589’, ‘Fletcher’, ‘Red Morning’, ‘Richmond’, ‘Summerpick’, and ‘Tasti Lee’), the performance ranking in relation to marketable yield did not change or only slightly changed when the plants were grafted with ‘Maxifort’ rootstock. Conversely, ‘Red Deuce’ and ‘Skyway’ improved their ranking after they were grafted with ‘Maxifort’ rootstock, further indicating that they are highly compatible. Finally, ‘Primo Red’ and ‘Scarlet Red’ had lower rankings when they were grafted with ‘Maxifort’ than as nongrafted plants, which suggests a lower degree of compatibility.

While it is clear that cultivar-to-cultivar differences exist in response to ‘Maxifort’ rootstock, the role of compatibility in the creation of these differences may be overstated somewhat in the literature. For this reason, it is difficult to determine which measurements are truly measuring graft compatibility and which are simply measuring the non-synergistic benefit of grafting plants with varying degrees of natural vigor onto a vigorous rootstock. The interactions between rootstock and scion likely involve compatibility, but must also be considered in the context of the environment (Albacete et al., 2015, Leal-Fernández et al., 2013). We used a high tunnel for our study due to the practical application of grafting technology and its prevalence in high tunnel production. “Good” (highly productive, low stress) environments typically encourage the expression of full genetic potential and allow for a more accurate genotype-to-genotype assessment (Hayward et al., 1993). However, especially given the significant year-to-year performance differences we observed, we cannot say that our environment was objectively “ideal” and the environmental conditions we provided likely acted as a limiting factor for the expression of the genetic potential in our rootstock x scion interactions. For a given set of environmental conditions, there may be a genetic limit to the productive advantage that a rootstock can provide.

Successful Combinations for High Tunnel Production

Many of the varieties that were utilized in this study have been used in nongrafted tomato variety trials (Egel, Zandstra, and Maynard, 2008; Goldy and Wendzel, 2015; Jones et al., 2007) or have been tested by others for high tunnel or greenhouse production (Au, 2011; Maynard and Bluhm, 2018; Reid, Klotzbach, Hoover, and Mattson, 2013; Rogers and Wszelaki, 2012; Wszelaki and Rogers, 2009). According to previously published variety trial reports, ‘Primo Red’ (Oxley and Rivard, 2015, 2016; Rogers and Wszelaki, 2012; Wszelaki and Rogers, 2009) and ‘Red Deuce’ (Oxley and Rivard, 2015, 2016; Maynard and Bluhm, 2018; Rivard et al., 2014b) are consistently high-yielding varieties. ‘BHN 589’ typically also yields well, placing in the top half in every trial it was in (Maynard and Bluhm, 2018; Oxley and Rivard, 2015, 2016; Rivard et al., 2014b; Rogers and Wszelaki, 2012; Wszelaki and Rogers, 2009). ‘Scarlet Red’ (Oxley and Rivard, 2015, 2016; Rivard et al., 2014b) and ‘Tasti Lee’ (Oxley and Rivard, 2015, 2016) often performed near the middle or bottom in their respective trials. ‘Summerpick’ was in only one trial in the literature, but in terms of yield, performed comparably to ‘Red Deuce’ and ‘BHN 589’ (Maynard and Bluhm, 2018). This contradicts the results of our study, for which ‘Summerpick’ was the third lowest performing variety (Table 2.5). For trials reported in the literature, ‘Fletcher’ consistently performed at a nearly or slightly above average level (Oxley and Rivard, 2016; Rogers and Wszelaki, 2012; Wszelaki and Rogers, 2009), though for our trial ‘Fletcher’ performed below average. With the exceptions of ‘Summerpick’ and (to a lesser extent) ‘Fletcher’, nongrafted yield data for our study showed very comparable trends (Table 2.5) to previous studies.

The combinations that we tested here span a range of 10.7 to 23.0 lbs of marketable fruit yield per plant when averaged across the two growing seasons. This wide range in our yield data should be useful to growers that are interested in any of the twenty grafted combinations or nongrafted cultivar options that we tested. ‘Red Deuce’ grafted onto ‘Maxifort’ rootstock showed a high degree of compatibility and produced the highest marketable (and total) yield of any variety we tested (Table 2.5). ‘Red Deuce’, ‘Red Morning’, ‘BHN 589’, ‘Primo Red’, and ‘Tasti Lee’ all provided at least 20 lbs of marketable fruit per plant when grafted onto ‘Maxifort’. For this reason, growers that are already utilizing these scion cultivars for high tunnel production should consider grafting their tomatoes onto ‘Maxifort’ rootstock to improve productivity.

Interestingly, nongrafted ‘Primo Red’ and ‘Red Morning’ were also ranked in the top half of the twenty different treatments tested and had higher fruit yield than several grafted varieties, (Table 2.4). Despite its lack of graft-induced performance benefit, nongrafted ‘Primo Red’, in particular, may have potential for commercial high tunnel production. Nongrafted ‘Primo Red’ was ranked sixth overall by marketable yield and produced 19.2 lbs of marketable fruit per plant (Tables 2.3 and 2.5). It was the highest yielding nongrafted variety and performed at a level comparable to many of the grafted varieties in terms of both marketable and total yield. ‘Primo Red’ may therefore be an excellent option for commercial high tunnel growers who do not wish to use grafted plants.

Conclusions

To our knowledge, this is the first report that examines the impact of scion on the benefit of grafting with ‘Maxifort’ rootstock. We found that implementing ‘Maxifort’ had a significant positive effect on the marketable yields of ‘BHN 589’, ‘Red Deuce’, ‘Skyway’, and ‘Tasti Lee’, in a high tunnel setting whereas ‘Primo Red’, ‘Red Morning’ and ‘Scarlet Red’ did not have a significant effect. We did not see a statistically significant interaction effect between graft-status and scion. Future work in this area would benefit from utilizing multiple rootstocks to better explore interactions that occur between rootstock and scion genotypes. An interesting approach for future research would be to compare high- and low-yielding varieties that have undergone reciprocal grafting to further elucidate the complex relationship that may occur between rootstock and scion.

Compatibility has been identified by sets of physiological traits that separate compatible from incompatible graft pairings (Edelstein, 2004; Gainza et al., 2015; Goldschmidt, 2014; Hartmann et al., 2013b). However, compatibility probably exists on a spectrum, and there is limited information in the literature that examines relative rootstock and scion compatibility quantitatively. We used two different methods to quantitatively assess compatibility across the ten scions that we tested. We were able to identify compatible, moderately compatible, and highly compatible scion varieties when they were grafted onto ‘Maxifort’ rootstock. By normalizing plant yield data to percent change, we can quantitatively assess the ability of the grafted plant to increase productivity over the nongrafted ones. We also ranked nongrafted and grafted performance of ten scion varieties and compared the shift that may or may not occur once grafting with ‘Maxifort’ was implemented. The goal of this process was to look at the scion genotype within each population independently to find out how the implementation of ‘Maxifort’ rootstock affected the individual plant performance of a scion amongst its “peers”. Although our population set is small, limiting the power of our statistical methods, plant breeders may consider using this same approach as they develop rootstock and scion combinations that are useful and economically-feasible (Rivard et al., 2010a; Rysin et al., 2015) for fresh-market tomato production.

Grafting with ‘Maxifort’ rootstock has the potential to significantly improve the yield, fruit size, and vegetative production of a wide variety of hybrid determinate tomato cultivars. Varieties like ‘BHN 589’, ‘Red Deuce’, ‘Skyway’, and ‘Tasti Lee’ showed significant benefits from grafting on ‘Maxifort’ rootstock in our study. Growers that are already utilizing these cultivars should consider grafting their tomatoes with ‘Maxifort’ rootstock to improve the productivity and profitability of their operations (Rivard et al., 2010a; Rysin et al., 2015). ‘Red Deuce’ grafted onto ‘Maxifort’ rootstock produced the highest marketable and total yields of any of the combinations that were tested. Other varieties like ‘Primo Red’ may still be useful for commercial production, though the benefit of grafting may be negligible. As researchers and growers in the U.S. continue to explore the use of grafted tomatoes for fresh-market high tunnel production, identifying combinations of rootstock and scion that maximize performance will be critical. This study provides an overview of how scion genotype can impact the benefit of grafting with vigorous rootstocks under little disease pressure and works to identify specific combinations that are highly compatible.

Tables

Table 2.1 - Disease resistance (as reported by seed companies) for determinate tomato scion and vegetative rootstock varieties used in a tomato scion grafting trial conducted in 2016 and 2017 in a high tunnel in Olathe, KS. Resistance codes based on (Johnny's Selected Seeds, 2018b)

		Fungus										Virus					Nematode
Scion	Seed Company	AS	AB	PL	F1	F2	F3	FOR	L	V1	V2	TMV	ToMV	ToANV	TSWV	TYLCV	N
BHN 589	BHN Seed				R	R				R*	R*	R					R*
Fletcher	Bejo Seeds									R*	R*				R		R*
Primo Red (HMX 7838)	Harris Moran				R	R				R	R		R		IR		
Red Deuce	Harris Moran		R		R	R			R	R			IR				
Red Morning	Harris Moran				R	R				R	R		R		IR		
Richmond	Syngenta	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
Scarlet Red	Harris Moran	R			R	R			R	R							
Skyway	Enza Zaden				R	R	R			R	R			R	IR	IR	IR
Summerpick	Syngenta				R	IR			R	R	R				R		
Tasti Lee	Bejo Seeds				R	R	R		R	R*	R*						
Rootstock																	
Maxifort	De Ruiter			R	R	R	R	R		R	R		R				IR

* = Race(s)/Species Not Specified x = Information Not Available ('Richmond' may no longer be commercially available) R = Resistant IR = Intermediate Resistance / Tolerance

Identified Resistance Traits include: Alternaria Stem Canker *Alternaria alternate* f. sp *lycopersici* (AS), Tomato Early Blight *A. solani* (AB), Corky Root Rot *Pyrenochaeta lycopersici* (PL), Fusarium Wilt *Fusarium oxysporum* f. sp. Lycopersici Races 1 (F1), 2 (F2), and 3 (F3), Fusarium Crown and Root Rot *F. oxysporum* f. sp. *radicis–lycopersici* (FOR), Gray Leaf Spot *Stemphylium solani* (L), Verticillium Wilt *Verticillium albo-atrum* (V1) and *V. dahliae* (V2), Tobacco Mosaic Virus (TMV), Tomato Mosaic Virus (ToMV), Tomato Apex Necrosis Virus (ToANV) Tomato Spotted Wilt Virus (TSWV), Tomato Yellow Leaf Curl Virus (TYLCV), and Root Knot Nematodes *Meloidogyne arenaria*, *M. incognita*, and *M. javanica* (N)

Seed Companies Included: Bejo Seeds: Oceano, CA, BHN Seed: Immokalee, FL, De Ruiter (Monsanto): Bergschenhoek, The Netherlands, Enza Zaden: Salinas, CA, Harris Moran: Modesto, CA, and Syngenta: Basel, Switzerland

Table 2.2 - *P*-values of main and interaction effects for: year, graft-status, and scion on marketable and total fruit yield for a tomato scion grafting trial conducted in 2016 and 2017 in a high tunnel in Olathe, KS. Data was transformed by year using the z-score standardization procedure prior to analysis.

Effect	Marketable fruit yield			Total fruit yield			Graft-induced change in mkt weight		Graft-induced change in total weight		Biomass (g)	
	Wt (lbs)	No.	Size (lbs)	Wt (lbs)	No.	Size (lbs)	% Change	Total change (lbs)	% Change	Total change (lbs)	Above-Ground	Below-Ground
Year	-	-	-	-	-	-	-	-	-	-	NA	NA
Graft Status	<.0001	<0.01	<.0001	<.0001	<.01	<.0001	NA	NA	NA	NA	<.0001	<.0001
Scion	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.05	<.05	0.1465	0.317	<.001	<.05
Year*Graft Status	0.1873	<.05	<.05	0.2655	<.05	<.01	NA	NA	NA	NA	NA	NA
Year*Scion	-	-	<.01	-	-	<.01	-	-	-	-	NA	NA
Graft Status*Scion	0.3319	0.0601	-	-	-	-	NA	NA	NA	NA	0.3707	-
Year*Graf-Status*Scion	-	-	-	-	-	-	NA	NA	NA	NA	NA	NA

The listed *P*s were determined using the General Linear Model procedure (SAS Studio: University Edition V 9.4; SAS Institute Inc. Cary, NC). *P*-values above 0.40 are not shown.

Table 2.3 - Simple effects of scion and graft-status on marketable and total fruit yield and size for grafted and nongrafted tomatoes grown in a high tunnel in 2016 and 2017 in Olathe, KS.

Scion[z]	Graft status[z]	Mkt yield (lbs/plant) 2016-2017	Total yield (lbs/plant) 2016-2017	Marketable yield (fruit no./plant)		Total yield (fruit no./plant)		Marketable fruit size (lbs/fruit)		Total fruit size (lbs/fruit)	
				2016	2017	2016	2017	2016	2017	2016	2017
BHN 589	Grafted	21.4 ab[y]	27.0 ab[y]	42.8 a[y]	44.5 a[y]	54.7 a[y]	61.6 a[y]	0.46 bcd[y]	0.49 defg[y]	0.44 bcdefg[y]	0.45 defgh[y]
	Nongraft	14.0 cdefg	19.2 b*cd	35.3 abc	35.8 ab	50.3 ab	54.6 ab	0.37 de	0.42 gh	0.34 hi	0.39 h
Fletcher	Grafted	17.8 abcde	22.0 abc	38.6 abc	41.9 ab	50.4 ab	55.1 ab	0.41 cde	0.47 efgh	0.39 defghi	0.44 efgh
	Nongraft	12.3 efg	14.7 cd	34.2 abc	34.5 ab	43.1 ab	43.8 ab	0.33 e	0.41 h	0.32 i	0.38 h
Primo Red	Grafted	21.0 ab	26.5 ab	32.2 abc	39.2 ab	41.0 ab	55.0 ab	0.54 ab	0.61 abcd	0.52 ab	0.56 abc
	Nongraft	19.2 abcd	22.1 abc	40.8 ab	37.2 ab	47.2 ab	47.3 ab	0.41 cde	0.57 bcde	0.40 cdefghi	0.53 bcde
Red Deuce	Grafted	23.0 a	27.6 a	32.3 abc	35.6 ab	39.1 ab	48.4 ab	0.60 a	0.70 a	0.58 a	0.64 a
	Nongraft	15.9 bcdefg	19.5 a*bcd	32.8 abc	26.2 b	40.4 ab	36.8 b	0.45 bcde	0.65 abc	0.43 bcdefgh	0.59 ab
Red Morning	Grafted	21.6 ab	27.3 ab	34.3 abc	37.1 ab	46.1 ab	50.6 ab	0.51 abc	0.67 ab	0.49 abcd	0.61 ab
	Nongraft	17.1 abcdefg	20.4 abcd	35.4 abc	34.3 ab	43.8 ab	46.1 ab	0.41 cde	0.58 bcde	0.38 efghi	0.53 bcdef
Richmond	Grafted	15.5 bcdefg	19.2 bcd	23.5 c	35.3 ab	31.9 b	44.8 ab	0.53 ab	0.54 cdefg	0.50 abc	0.51 bcdefg
	Nongraft	10.9 fg	12.6 d	27.2 bc	29.7 ab	32.8 ab	36.2 b	0.39 de	0.41 h	0.38 efghi	0.39 h
Scarlet Red	Grafted	16.4 bcdefg	20.7 abcd	29.7 abc	34.3 ab	39.8 ab	45.7 ab	0.47 bcd	0.55 bcdef	0.45 bcdef	0.51 bcdefg
	Nongraft	13.4 defg	17.2 cd	31.4 abc	37.3 ab	43.5 ab	47.9 ab	0.36 de	0.44 fgh	0.35 ghi	0.41 gh
Skyway	Grafted	17.3 abcdef	23.0 abc	28.3 abc	32.5 ab	42.6 ab	45.0 ab	0.52 abc	0.60 abcd	0.48 abcde	0.56 abcd
	Nongraft	10.7 g	15.6 cd	23.6 c	28.2 b	40.5 ab	39.9 b	0.41 cde	0.45 efgh	0.37 fghi	0.42 fgh
Summerpick	Grafted	16.8 abcdefg	23.0 abc	24.5 c	34.9 ab	36.9 ab	49.1 ab	0.54 ab	0.60 abcd	0.50 abc	0.56 abcd
	Nongraft	12.1 efg	15.6 cd	24.6 c	30.8 ab	34.2 ab	40.1 b	0.43 bcde	0.47 efgh	0.41 cdefghi	0.45 efgh
Tasti Lee	Grafted	20.1 abc	22.8 abc	40.5 ab	45.0 a	46.7 ab	55.4 ab	0.44 bcde	0.50 defgh	0.42 bcdefghi*	0.47 cdefgh
	Nongraft	12.5 efg	14.8 cd	33.2 abc	34.2 ab	41.2 ab	44.1 ab	0.33 e	0.43 fgh	0.32 i	0.40 h

[z] The experimental design was a randomized complete-block design with four replications. Plots consisted of five plants of either the grafted or nongrafted scion cultivars listed above. All grafted scions utilized 'Maxifort' rootstock. In row spacing was 18”, and tomatoes were harvested at first blush approximately every 5-7 days for 91 days between late June and early October both years, and the cumulative per-plant harvest data was included in the statistical analysis. Data was transformed across years using the z-score transformation prior to evaluation and back-transformed for presentation

[y] Values representing the back-transformed means marked with the same letter do not differ significantly using Tukey’s Multiple Comparisons Adjustment at α=0.05.

* *P*-values for a significant difference between nongrafted varieties and corresponding grafted plants fall within a 90% confidence interval, but are not significant for a 95% confidence interval

Table 2.4 - Actual and percent change in marketable yield of ten tomato varieties due to grafting with ‘Maxifort’ rootstock when grown in a high tunnel in 2016 and 2017 in Olathe, KS.

Scion[z]	Change in mkt yield (lbs/plant)	Ranking	% Change in mkt yield		Ranking
Tasti Lee	7.04 [y]	1	59.5%	a[y]	1
BHN 589	6.86	2	47.8%	ab	3
Red Deuce	6.42	3	41.0%	ab	5
Skyway	6.00	4	56.4%	ab	2
Fletcher	5.01	5	42.5%	ab	4
Summerpick	4.24	6	36.6%	ab	7
Richmond	4.13	7	39.7%	ab	6
Red Morning	4.05	8	25.1%	ab	8
Scarlet Red	2.73	9	20.9%	ab	9
Primo Red	1.47	10	9.2%	b	10

[z] The experimental design was a randomized complete-block design with four replications. Plots consisted of five plants of either the grafted or nongrafted scion cultivars listed above. All grafted scions utilized 'Maxifort' rootstock. In row spacing was 18”, and tomatoes were harvested at first blush approximately every 5-7 days for 91 days between late June and early October both years, and the cumulative per-plant harvest data was included in the statistical analysis.

% change in mkt yield was calculated as: % change = [(grafted yield/nongrafted yield) – 1] x 100

[y] Data was transformed across years using the z-score transformation prior to evaluation and back-transformed for presentation. Values representing the back-transformed means marked with the same letter do not differ significantly using Tukey’s Multiple Comparisons Adjustment at α=0.05. No values for the "Change in Mkt Yield (lbs/plant)” showed statistically significant differences though ‘Skyway’ showed a nearly significant percent change in marketable yield as compared to ‘Primo Red’ ($P = 0.0572$)

Ranked data is organized from highest value (rank of 1) to lowest value (rank of 10) irrespective of the significance level of the difference.

Table 2.5 - Ranked mean values for marketable and total yield for ten grafted and nongrafted tomato varieties grown in a high tunnel in 2016 and 2017 in Olathe, KS.

	Marketable yield (lbs/plant) rankings				Total yield (lbs/plant)			
	Ranked separately (1-10)		Ranked together (1-20)		Ranked separately (1-10)		Ranked together (1-20)	
Scion[z]	Nongraft	Grafted	Nongraft	Grafted	Nongraft	Grafted	Nongraft	Grafted
Primo Red	1	4	6	4	1	4	8	4
Red Morning	2	2	9	2	2	2	11	2
Red Deuce	3	1	12	1	3	1	12	1
BHN 589	4	3	14	3	4	3	13	3
Scarlet Red	5	9	15	11	5	9	15	10
Tasti Lee	6	5	16	5	8	7	18	7
Fletcher	7	6	17	7	9	8	19	9
Summerpick	8	8	18	10	6	6	16	6
Richmond	9	10	19	13	10	10	20	14
Skyway	10	7	20	8	7	5	17	5

Data was averaged across both years of the study, and ranking values for each category is displayed both separated by graft status, and together. Categories are ranked from highest value (rank of 1) to lowest value (rank of 10) irrespective of the significance level of the difference.

[z] The experimental design was a randomized complete-block design with four replications. Plots consisted of five plants of either the grafted or nongrafted scion cultivars listed above. All grafted scions utilized 'Maxifort' rootstock. In row spacing was 18”, and tomatoes were harvested at first blush approximately every 5-7 days for 91 days between late June and early October both years, and the cumulative per-plant harvest data was included in the statistical analysis.

Table 2.6 - Tukey's honest significant difference test for above and below-ground dry biomass as effected by graft status and scion variety for a tomato grafting trial conducted in 2017 in a high tunnel in Olathe, KS

Scion[z]	Graft status[z]	Above-ground biomass (g)		Below-ground biomass (g)	
BHN 589	Grafted	380.6	defg[y]	12.94	ab[y]
	Nongraft	188.6	ab	8.73	abc
Fletcher	Grafted	312.2	bcdefg	11.41	abc
	Nongraft	191.8	ab*	8.68	abc
Primo Red	Grafted	288.5	abcdefg	8.48	abc
	Nongraft	238.6	abcd	7.03	c
Red Deuce	Grafted	346.7	cdefg	9.62	abc
	Nongraft	245.7	abcdef	7.34	c
Red Morning	Grafted	417.5	g	11.83	abc
	Nongraft	267.2	abcdefg	9.19	abc
Richmond	Grafted	394.6	defg	11.53	abc
	Nongraft	210.9	abc	7.84	abc
Scarlet Red	Grafted	380.2	defg	11.27	abc
	Nongraft	268.6	abcdefg	7.69	bc
Skyway	Grafted	276.4	abcdefg	10.85	abc
	Nongraft	172.5	a	8.62	abc
Summerpick	Grafted	409.8	fg	13.29	a
	Nongraft	242.8	abcde	9.12	abc
Tasti Lee	Grafted	401.8	efg	11.66	abc
	Nongraft	275.3	abcdefg	8.01	abc

[z] Experimental design was a randomized complete-block design with four replications. Plots had five plants of either the grafted or nongrafted scions listed above. All grafted scions utilized 'Maxifort' rootstock. In row spacing was 18" At the end of the 91 day harvest season, two plants from the center of each plot were destructively sampled to collect above and below-ground biomass data. Above-ground biomass samples were taken by cutting the stem at approximately ground level. Below-ground biomass samples were collected using a shovel to dig out as much of the root ball as possible, and then washing the roots prior to drying. Prior to being weighed, both above and below-ground biomass samples were dried using a "Grieve Industrial Shelf Batch Oven" model SC-400 (The Grieve Corporation, Round Lake, Illinois) at 160oF (71.1oC) for approximately 6 days (144 h) with the blower on. Per-plant biomass data for each plot was calculated as the simple average for the two samples.

[y] Data was transformed logarithmically prior to evaluation and back-transformed for presentation. Values representing the back-transformed means marked with the same letter do not differ significantly using Tukey's Multiple Comparisons Adjustment at α=0.05.

**P* values for a significant difference between nongrafted varieties and corresponding grafted plants fall within a 90% confidence interval, but are not significant for a 95% confidence interval. *P* = 0.0921 for the difference in above-ground biomass between grafted and non-grafted 'Fletcher'

Table 2.7 - Correlations between biomass and yield for a tomato grafting trial conducted in 2017 in a high tunnel in Olathe, KS (N=80)

		Yield (no of fruit per plant)		**Yield (lbs of fruit per plant)**		**Fruit size (lbs/fruit)**	
		Marketable	**Total**	**Marketable**	**Total**	**Marketable**	**Total**
Above-ground dry biomass (g)	Pearson correlation coefficient	0.39663	0.42124	0.54963	0.5484	0.35941	0.34817
	P-value for relationship	0.0003	<.0001	<.0001	<.0001	0.0011	0.0016
Below-ground dry biomass (g)	Pearson correlation coefficient	0.41842	0.48076	0.39885	0.42793	0.11532	0.10156
	P-value for relationship	0.0001	<.0001	0.0002	<.0001	0.3084	0.37

The experimental design was a randomized complete-block design with four replications. Plots consisted of five plants of either the grafted or nongrafted scion cultivars listed above. All grafted scions utilized 'Maxifort' rootstock. In row spacing was 18”, and tomatoes were harvested at first blush approximately every 5-7 days for 91 days between late June and early October. At the end of the season two plants from the center of each plot were destructively sampled to collect above and below-ground biomass data. Above-ground biomass samples were taken by cutting the stem at approximately ground level. Below-ground biomass samples were collected using a shovel to dig out as much of the root ball as possible, and then washing the roots prior to drying. Prior to being weighed, both above and below-ground biomass samples were dried using a “Grieve Industrial Shelf Batch Oven” model SC-400 (The Grieve Corporation, Round Lake, Illinois) at 160oF (71.1oC) for approximately 6 days (144 h) with the blower on. Per-plant biomass data for each plot was calculated as the simple average for the two plants sampled.

Chapter 3 - Evaluating *Solanum* and *Capsicum* Rootstocks for Fresh-Market Bell Pepper Production

Summary

While the adoption of grafting for pepper production is increasing globally, most grafted peppers are used in tropical or subtropical areas to counter the effects of disease or abiotic stress common to those climates. Because most pepper rootstocks have been developed primarily for tolerance/resistance to disease and abiotic stress, it is unclear whether grafting with these rootstocks can provide a significant yield advantage over nongrafted plants for growers in the U.S. with relatively few disease or stress-related barriers to production. Additionally, compared to rootstocks for tomatoes, relatively few rootstocks have been developed for use with peppers, and there are similarly few reports of research on grafted peppers available. There are conflicting reports in the literature regarding the compatibility of intergeneric *Capsicum*/*Solanum* grafts. However, if the two prove compatible, the use of solanaceous rootstocks for pepper production may have the potential to increase plant vigor and disease resistance.

The objectives of this study were to identify commercially-available pepper rootstocks that improve crop productivity and determine the viability of using solanaceous rootstocks for bell pepper production. We conducted five trials in 2016 and 2017 that utilized a randomized complete block design with at least 3 replications. ‘Karisma’ was the nongrafted control and was used as scion for three *Capsicum* rootstocks (‘Scarface’, ‘Yaocali’, and ‘Meeting’) in addition to two *Solanum* rootstocks (‘Maxifort’ and ‘Sweetie’). We found that ‘Scarface’ rootstock

significantly improved yield ($P < 0.05$) and plants grafted with 'Scarface' had on average: 32% greater marketable yield, 15%-18% larger fruit, and 9-12% higher marketability than the nongrafted 'Karisma' plants. While the trends for plants grafted with 'Yaocali' were similar to those grafted to 'Scarface', they were less consistent and there were fewer statistically significant differences when compared to nongrafted plants. 'Meeting' rootstock did not have any significant effects on yield compared to the nongrafted plants, and 'Meeting' may be more useful for managing diseases than improving yield in situations with low disease pressure. We also observed significant correlations between plant biomass and yield, indicating that for the future development of high-yielding pepper rootstocks, researchers and plant breeders should consider the use of highly vegetative pepper accessions. The solanum rootstocks, 'Maxifort' and 'Sweetie', displayed symptoms of delayed incompatibility when grafted with 'Karisma' scions, including significant (78% to 89%) reductions in yield and (59% to 93% less) biomass. Pepper plants grafted to these rootstocks also exhibited malformations at the graft union and higher in-field mortality rates, possibly due to the increased lignification that occurs in pepper stems. Based on our results, *Solanum* rootstocks are not suitable for bell pepper production, but the utilization of *Capsicum* rootstocks, 'Scarface' and 'Yaocali' may be useful for growers that want to increase crop productivity, and the development of more rootstocks like these may help to improve the adoption of pepper grafting in the United States.

Introduction

Vegetable grafting has been used in greenhouse and high tunnel production systems worldwide (Kubota et al., 2008). In (soil-based) high tunnel systems, certain problems including soil quality issues and increased pressure from certain diseases may be caused or aggravated by year-round intensive cultivation (Reeve and Drost, 2012) and limited crop rotation (Carey et al., 2009; Galinato and Miles, 2013; Knewtson, 2008; Lamont, 2009; Reeve and Drost, 2012). Producers are turning to grafted plants to address these issues and increase crop productivity,

Many vegetable rootstocks have been selected for disease resistance or tolerance (Albacete et al., 2015; Davis et al., 2008a; Rodriquez and Bosland, 2010). Grafting onto such rootstocks can be an effective disease management tool (Bletsos and Olympios, 2008; Blomgren and Frisch, 2007; Edelstein, 2004; Fernández-García et al., 2004; Lee, 1994; Louws et al., 2010, 2011; Michel et al., 2015; Oda, 1999; Rivard and Louws, 2008; 'Tomato Grafting', 2015). Grafting onto certain rootstocks can also improve plant tolerance of harsh growing conditions and may help to address soil quality issues (Reeve and Drost, 2012). Such traits include: thermal stress tolerance found in *Solanum* rootstocks (Bletsos and Olympios, 2008; Edelstein, 2004), high soil salinity tolerance found in tobacco rootstocks (Haberal et al., 2016), and waterlogging/flooding tolerance found in a variety of eggplant (*S. melongena*, and *S. torvum*) and pepper (*Capsicum annum*) rootstocks (Bahadur et al., 2015; Petran, 2013; Schreinemachers and Afari-Sefa, 2012; SATNET Asia, 2015; Wu et al., 2012).

The use of rootstocks of a different species or genera can also help to address these goals. Such interspecific grafting is practiced for eggplant and tomato (Haberal et al., 2016), and can help to provide verticillium wilt resistance for eggplant (by grafting onto tomato rootstocks) (Petran, 2013), and cold or flooding resistance for tomato (by using eggplant rootstocks) (Bahadur et al., 2015; Petran, 2013). Both interspecific and intergeneric grafting may be used to obtain non-host resistance, which is more durable than other forms because it involves more genes and prevents the adaptation of a pathogen to the organism (Gill et al., 2015). Such grafting has been used to manage fusarium wilt (*F. oxysporum f. sp. cucumerinum*, *lagenariae, melongenae,* and *melonis*) in cucurbits (Louws et al., 2010). Intergeneric grafting between solanaceous species like tomato and tobacco has also been reported to improve yield and plant growth (Haberal et al., 2016).

Even in the absence of pressure from disease or abiotic stress the implementation of certain rootstocks (not only intergeneric ones) has the potential to improve the overall health and vigor of solanaceous plants (Albacete et al., 2015; Djidonou et al., 2013; Haberal et al., 2016; Lee, 1994). Because they are better positioned to express their full genetic potential many grafted plants are especially productive in low-stress environments (Hayward et al.,1993), leading to improvements in both yield (Bletsos and Olympios, 2008; Davis et al., 2008b; Djidonou et al., 2013; Edelstein, 2004) and fruit marketability (Davis et al., 2008b; Frey et al., 2017; Haberal et al., 2016).

Pepper Grafting

While the use of grafted peppers is increasing in Asia (Lee, 2003; Wu et al., 2012), their use in the United States still lags far behind other grafted solanaceous crops like tomatoes (Kubota et al., 2008). The website vegetablegrafting.org (2017) currently lists only five commercially-available pepper rootstocks, as compared to 48 commercially available tomato rootstocks. While not an exhaustive list, this disparity is a strong indication that there are comparatively few pepper rootstocks available. Many pepper rootstocks are interspecific hybrids of *C. annum* x *C. chinense*, or are cultivars of *Capsicum* species including *C. annum*, *C. baccatum*, *C. chinense*, and *C. frutescens* (Bletsos and Olympios, 2008).

Most pepper rootstocks have been developed primarily for disease resistance and abiotic stress tolerance (Leal-Fernández et al., 2013; Petran, 2013). Pepper rootstocks have been identified for managing tobacco mosaic virus (Bletsos and Olympios, 2008), phytophthora blight (*Phytophthora capsici*) (Bletsos and Olympios, 2008; Sen et al., 2018), verticillium wilt (*Verticillium dahliae*) (Bletsos and Olympios, 2008), and nematodes (Bletsos and Olympios, 2008; Michel et al., 2015; Sen et al., 2018). There are also reports of rootstocks reducing the effects of abiotic stressors on pepper plants such as: flooding (Sen et al., 2018; Wu et al., 2012), drought stress (Sen et al., 2018), and high heat (Petran, 2013; Sen et al., 2018). Rootstocks that confer disease resistance and tolerance to abiotic stress are especially important in tropical climates like Southeast Asia, where these problems are a major hindrance to production (Wu et al., 2012). However, outside of the tropics, where disease and abiotic stress is less of a problem, pepper grafting has seen little use (Michel et al., 2015). Although pepper rootstocks may significantly improve yields when there are identified stressors in the production system (Penella and Nebauer, 2014), this focus on biotic and abiotic stress tolerance/resistance during the development of many of these rootstocks may have resulted in a lack of vigor amongst pepper rootstocks, especially compared to rootstocks used in tomato grafting, making them less practical when disease and/or abiotic stress are not major problems (Leal-Fernández et al., 2013).

Pepper rootstocks can significantly influence morphological and production-related traits (Leal-Fernández et al., 2013) and some vigorous pepper rootstocks, specifically interspecific hybrids like *C. annum* x *C. chinense* are reported to significantly improve productivity (Bletsos and Olympios, 2008). These rootstocks may display a “generative” growth pattern, with grafted plants allocating more resources to the production of fruit and moderating vegetative growth (Albacete et al., 2015). Similarly, in tomato, ‘Maxifort’ is reported to grow more vigorously than rootstocks like ‘Trooper Lite’. While both rootstocks provide significantly increased yield, the plants grafted with ‘Maxifort’ have dramatically higher vegetative biomass whereas plants grafted with ‘Trooper Lite’ display a generative growth pattern (Masterson et al., 2016). While generative pepper rootstocks may increase efficiency by allowing for increased planting density, and by reducing costs associated with chemical and fertilizer use (Albacete et al., 2015), several studies with grafted peppers have correlated plant growth with productivity (Leal-Fernández et al., 2013; Soltan, et al., 2017). Root size was one trait that was found to be positively correlated with yield, shoot biomass, and stem diameter in grafted peppers (Leal-Fernández et al., 2013). Similarly, peppers grafted with certain commercial rootstocks had higher yield than nongrafted ones and exhibited greater vegetative growth (Soltan et al., 2017). For this reason, the development of highly compatible "vegetative" rootstocks may provide potential for significant improvements in yield even when disease or environmental stressors are not present. Due to the potential for a wide range of grafting responses when using pepper rootstock, it is important to select stock-scion combinations with specific growing conditions and challenges in mind (Leal-Fernández et al., 2013).

Louws et al., (2010) proposed that grafting may also be a viable to method for managing Phytophthora crown and root rot (caused primarily by *P. capsici*) in pepper. This disease causes significant losses for large-scale pepper producers in the United States (Pernezny et al, 2003) (Foster and Hausbeck, 2010; Koike et al., 2016; Sanogo and Carpenter, 2006; 2007) . However, while some "tolerant" pepper lines exist, none display complete resistance to *P. capsici*, (Foster and Hausbeck, 2010; Koike et al., 2016; Louws et al., 2010; Mo et al., 2014; Parada Rojas et al., 2015). Non-host resistance genes for *P. capsici* have been discovered in tobacco (Koike et al., 2016), and while tomatoes can serve as a host to *P. capsici* (Louws et al., 2010; Parada Rojas and Quesada-Ocampo, 2015; Seminis Vegetable Seeds, 2016), the effect on the plants is minimal compared to pepper. Therefore, tomato rootstocks might confer disease tolerance that could be valuable for growers that are trying to manage *P. capsici*. Additionally, given the relatively small pool of vigorous pepper rootstocks that are commercially available, identifying rootstocks in related genera such as *Solanum* may be a feasible alternative to inter-specific *Capsicum* rootstocks for improving crop productivity.

Compatibility

One drawback of intergeneric grafting is the potential for graft incompatibility (Edelstein, 2004). Incompatible rootstock and scion combinations can negate many of the potential benefits of grafting, and have been reported in the literature for several vegetable crops including cucurbits (Davis et al., 2008a), tomatoes, eggplants (Oda et al., 2005) and peppers (Goldschmidt, 2014; Kawaguchi et al., 2008). However, reports in the literature are not clear regarding the feasibility and compatibility of using rootstocks from other solanaceous species for pepper production (Eltayb et al., 2013; Goldschmidt, 2014; Kawaguchi et al., 2008; Petran, 2013; Rodriquez and Bosland, 2010). Immediate graft incompatibility is characterized by the rapid death of the scion tissue and is attributed to the failure of the vascular tissue to heal and function properly (Mudge, 2013). Incompatibility may also be delayed, though it is still associated with recognizable symptoms, including growth inhibition, high leaf chlorophyll content, the production of undersized fruit, wilting, high rates of mortality (Kawaguchi et al., 2008; Oda et al., 2005), and scion over or under-growth (Gainza et al., 2015; Goldschmidt, 2014; Kawaguchi et al., 2008; Oda et al., 2005). While there is no precise definition for graft compatibility, there is a general consensus throughout the literature. Goldschmidt (2014) outlined three basic characteristics of compatible grafting partners: 1) The establishment of a successful graft union 2) The extended survival of the composite plant, and 3) The proper functioning of the composite, grafted plant. Edelstein (2004) and Gainza et al., (2015) also elaborated on these concepts when describing basic symptoms of incompatibility. These symptoms included a failure of the rootstock and scion to unite, a lack of health, the premature death of the plant caused by graft breakage or the failure of the graft union, and the inability for the grafting partners to form a strong, lasting, and functional union.

Predicting Compatibility Within the Solanaceous Family

The mechanisms involved in compatibility are not fully understood (Andrews and Marquez, 1993; Venema et al., 2011). However, graft compatibility probably has a strong genetic component that involves the movement and expression of genetic material between rootstock and scion (Albacete et al., 2015; Bletsos and Olympios, 2008; Lucas et al., 2001; Ruiz-Medrano et al., 1999; Velez-Ramirez et al., 2014; Zhang et al., 2014).

While tomato is most closely related to potato, followed by eggplant (Park et al., 2011; Särkinen et al., 2013; The Tomato Genome Consortium, 2012), the majority of the difference in genomic size between tomato and pepper can be attributed to differences in introns (Kim et al., 2014; Park et al., 2011) and studies of Tomato (*S. Lycopersicum*), Eggplant (*S. melongena*), and Pepper (*C. annum*) indicate that basic gene functions and number (~35,000) are conserved across species. In spite of the broad range of genome sizes, the order and function of genes (on each chromosome) is very similar from species-to-species within the Solanaceous family, with tomato and pepper genomes sharing large, well-conserved syntenic blocks (Kim et al., 2014; Park et al., 2011; Quiros, 2010; Rinaldi et al., 2016). For these reasons, tomato and pepper are considered to be relatively closely-related, more so than tomato and tobacco (Park et al., 2011; Rinaldi et al., 2016; Särkinen et al., 2013). Given the significant genetic component of compatibility, the relative phylogenetic proximities of tomato to pepper and tobacco, and the high degree of compatibility displayed by intergeneric tomato/tobacco grafts (Haberal et al., 2016), it is plausible that tomato and pepper plants could display a relatively high degree of intergeneric graft compatibility.

The earliest attempts at interspecific and intergeneric grafts within the solanaceous family reported successful tomato/potato, tomato/pepper, and pepper/eggplant grafts with equally successful reciprocal grafts (Rice, 1891). Many of these grafting combinations have since been reproduced with varying degrees of success. Tomato/potato (*Solanum tuberosum*) grafts are one of the most compatible and have been used to successfully produce both crops on the same plant (Kudo and Harada, 2007: Kumar, 2011). Tomato/eggplant (*Solanum* melongena) grafts (and their reciprocals) may be useful under certain conditions (Bahadur et al., 2015; Petran, 2013). However, they are considered “moderately incompatible” due to slight reductions in the yield of tomatoes when grafted onto eggplant rootstock (Goldschmidt, 2014; Kawaguchi et al., 2008; Oda et al., 2005). Additionally, while vascular irregularities were observed at the graft union in tomato when grafted onto eggplant rootstock by Oda et al., (2005), Kawaguchi et al., (2008) reported normal vascular anatomy in both tomato on eggplant, as well as reciprocal graft combinations. Even intergeneric tomato/tobacco grafts demonstrate a high degree of compatibility, displaying improved plant growth and high yields (Haberal et al., 2016). Recent reports have also discussed the successful grafting of tomatoes, peppers, and related species (Albacete et al., 2015; Eltayb et al., 2013; Petran, 2013; Rodriquez and Bosland, 2010). Furthermore, tomato accessions or hybrids that are cross-compatible (compatible with both tomato and pepper), have been identified (Albacete et al., 2015; Chetelat and Petersen, 2003; Petran, 2013). However, other studies have reported that reciprocal tomato/pepper grafts are "severely incompatible" (Goldschmidt, 2014; Kawaguchi et al., 2008), and failed attempts to graft peppers onto a variety of *Solanum* species (including *S. scabrum,* and *S. gilo*) suggest that peppers should only be grafted to rootstocks from within the *Capsicum* genus (Bletsos and Olympios, 2008). One report indicated that pepper plants grafted onto tomato rootstocks died prior to flowering, and that reciprocal grafts produced (at most) one fruit before dying (Kawaguchi et al., 2008). Other studies described symptoms of delayed incompatibility including growth inhibition, high leaf chlorophyll content, the production of undersized fruit, wilting, and high rates of mortality (Kawaguchi et al., 2008; Oda et al., 2005).

It is important to note that the apparent initial formation of callus tissue and a "healed" graft union does not guarantee the long-term success of the grafted plant. Often times incompatibility is "delayed" (Andrews and Marquez, 1993; Gainza et al., 2015 ; Mudge, 2013). One common symptom of delayed graft incompatibility is scion over or under-growth. These deformities are often indicative of malformations in the vascular tissue at the graft interface, which interferes with the exchange of water, minerals, and nutrients between stock and scion (Gainza et al., 2015; Goldschmidt, 2014; Kawaguchi et al., 2008; Oda et al., 2005). These malformations may also be caused by differences in growth patterns between scion and rootstock (Hartmann et al., 2013b), or by the accumulation of lignin and the formation of callus tissue within the graft union as a part of the plants wound response (Andrews and Marquez, 1993). In tomato, delayed incompatibility may be due to differences in viral resistance/tolerance between graft partners. This occurs when a grafting partner with no resistance will slowly succumb to a virus that migrates from the resistant/tolerant (and therefore asymptomatic) partner (Dogra et al., 2018; Mudge, 2013; Rosskopf et al., 2013, 2014; Rowhani et al., 2017).

Project Goals

The overall goal of this report was to evaluate pepper *(C. annum)* and tomato (*S. lycopersicum*) rootstocks for commercial bell pepper fruit production in the Central U.S. More specifically, the objectives were: 1) to determine whether grafting sweet bell peppers to commercially available pepper rootstocks is a viable method for increasing plant growth and/or crop productivity in growing systems with little pressure from disease or abiotic stress, and 2) to assess the compatibility and potential for intergeneric grafting by grafting bell pepper scion onto *Solanum* rootstocks.

Materials and Methods

A total of five trials were conducted at four sites in Kansas during 2016 and 2017. The trial sites, outlined below included two university research centers, a student-run organic farm at Kansas State University, and a privately owned commercial farm. All five trials used ‘Karisma’ (aka HMX 6646, Harris Moran Seed Co. Modesto, CA) as the scion and for nongrafted controls. All trials were planted in a randomized complete block design with at least three replications. Due to differences in available space and on-farm management practices, the trials varied from site-to-site in terms of replication number, plot size, cultural methods, and the rootstocks that were evaluated. As described below, yield data was converted to per-plant production prior to statistical analysis. One trial was located in the open-field, but the other four were located in high tunnels. Details for each trial are described below.

The treatments that were tested included nongrafted ‘Karisma’ as well as ‘Karisma’ grafted onto ‘Maxifort’, ‘Sweetie’, ‘Scarface’, ‘Yaocali’, and ‘Meeting’ rootstocks. Soltan et al., (2017) found that nongrafted plants had yields that were not statistically different from self-grafted plants, so additional self-grafted controls were not used in our study. ‘Karisma’ is an F1 hybrid sweet green to red (bell) pepper that produces large blocky, and uniform, thick-walled fruit. The variety is resistant to bacterial spot, Tobacco Mosaic Virus, Potato Virus Y, and Pepper Mottle Virus, with intermediate resistance to Cucumber Mosaic Virus. ‘Karisma’ is commonly used in research trials (Bayogan and Cantwell, 2011; Dunn et al., 2013; 2014; Foster and Hausbeck, 2010; Sanchez et al., 2011), and performs well in high tunnel production systems (Oxley et al., 2017; Oxley and Rivard, 2016, 2018; Rivard and Oxley, 2015; Rivard et al., 2014a; Wright, 2016).

The rootstocks that were tested included two cultivars from the *Solanum* genera: Maxifort and Sweetie. 'Sweetie' (Petoseed, Ventura, CA), is a vigorous open-pollinated indeterminate cherry tomato (*Solanum lycopersicum*) reportedly resistant to Alternaria Stem Canker. 'Maxifort' (De Ruiter/Monsanto, Bergschenhoek, The Netherlands) is widely-used as a rootstock for tomato production and is an interspecific hybrid (*S. lycopersicum* x *S. Habrochaites*) (McAvoy, 2005; Bletsos and Olympios, 2008; Buller et al., 2013; Masterson et al., 2016b ; Hu and Kleinhenz, 2015; Hu, 2016; Meyer, 2016). 'Maxifort' has been tested with numerous tomato scion varieties (AGIS, 2015), and typically increases plant growth, yield (Barrett et al., 2012; Djidonou et al., 2013; Frey et al., 2017; Masterson et al., 2016b; Rivard and Louws, 2008), and nitrogen and water use efficiency (Djidonou et al., 2013). 'Maxifort' is resistant to corky root, fusarium wilt (races 1-2) (Rivard and Louws, 2008), fusarium crown and root rot, southern blight (S. Rolfsii) (Rivard et al., 2010b), verticillium wilt (*V. dahliae*), and Tomato Mosaic Virus, and is tolerant of root-knot nematodes (*M. incognita*) (Rivard et al.,2010b).

The other three rootstocks used in this study are F1 *Capsicum* hybrids and included: 'Meeting' (Sakata, Morgan Hill, CA), 'Scarface' (Enza Zaden/Vitalis, Salinas, CA), and 'Yaocali' (Enza Zaden/Vitalis, Salinas, CA). 'Meeting' is resistant to Tobacco Mosaic Virus and has intermediate resistance to phytophthora root rot and corky root rot. 'Scarface' is resistant to

Tobamovirus (race 0) and has intermediate resistance to root knot nematodes (*Meloidogyne arenaria*, *M. incognita*, and *M. javanica*). ‘Yaocali’ (Enza Zaden/Vitalis, Salinas, CA) is resistant to Tobamovirus (races 0-3) and is tolerant of abiotic stressors including high salinity and flooding.

The goal of this study was to evaluate rootstocks for compatibility and associated changes in yield and plant morphology, therefore trials were not placed in areas that had a history of soilborne disease issues. All trials were grown in a stake-and-weave vertical trellis system with drip irrigation and utilized typical commercial practices in the region (Buller et al., 2016).

Transplant Production and Grafting

All grafted and nongrafted transplants were propagated at the Kansas State University Olathe Horticulture Research and Extension Center (Olathe, Kansas). The tube grafting technique, also known as splice grafting or Japanese top-grafting is common for tomatoes (MacDonald, 2014; McAvoy, 2005; Meyer, 2016; Oda, 1999; Rivard and Louws, 2006) and was utilized for all grafted pepper treatments. During the grafting procedure and just prior to grafting, approximately 80-90% of the scion leaf tissue was removed similar to Meyer et al., (2017) and Masterson et al., (2016). Rootstock and scion seedlings were grafted at the two to four true leaf stage and were held together for the duration of the healing process with 1.5 or 1.7 mm silicon grafting clips (Hydro-Gardens, Colorado Springs, CO).

Grafted seedlings were placed inside a healing chamber that was covered with a polyethylene film, 2-3 layers of 55% shade cloth, and a supplemental cool-mist humidifier. Healing chamber management protocols developed for tomatoes were generally followed (Rivard and Louws, 2011). However, grafted pepper transplants spent between 12 and 14 d in the healing chamber compared to 7-10 d for tomato. Following graft union formation, all grafted seedlings were removed from the healing chamber and grown in the greenhouse for at least 14 d before hardening off outdoors and transplanting.

Olathe Horticulture Research and Extension Center (OHREC)

High tunnel trials were planted in 2016 and 2017 at the Kansas State University Olathe Horticulture Research and Extension Center (OHREC), located in Johnson County (lat. 38.894406N, long. 94.995473W). However, excessive damage from wildlife in 2017 meant that all data from that trial was discarded. The soil type at this location was a chase silt loam (pH = 6.8). The 2016 trial was nested within a pepper variety trial covering four rows in one bay of a three season, multi-bay high tunnel (Haygrove, Ledbury, UK). One replication was planted within each of the four 126-ft rows. Plants grafted with ‘Maxifort’ and ‘Scarface’ rootstocks were compared to nongrafted ‘Karisma’. Each plot had six plants and in-row plant spacing was 18 inches with rows that were five ft apart. One empty space was left between plots. A raised-bed growing system was used, and weeds were suppressed via woven fabric mulch between beds and plastic mulch over the beds. Prior to planting, calcium nitrate (15.5-0-0) was applied at a rate of 50 lbs N/acre to the rows as a pre-plant fertilizer. Four applications of water-soluble nitrogen fertilizer were applied on a scheduled basis via fertigation at a rate of 10 lbs N/acre on 29 April, 1 and 30 June, and 1 Aug 2016. The first and third applications utilized potassium nitrate (13-0-46), while the second and fourth applications utilized calcium nitrate (13.5-0-0). The OHREC trial was planted on 27 April 2016. Harvesting occurred on 22 June, 1, 12, 22, and 29 July, 8, 17, and 26 August, and 7, 12, and 21 September.

John C Pair Horticulture Center (JCPHC)

Trials were conducted in 2016 and 2017 at the John C Pair Horticulture Center (JCPHC), located in Sedgwick County, KS (lat. 37.518064N, long. 97.310835W). The soil at this location consisted of a Canadian-Waldeck fine sandy loam (pH = 6.9). The 2016 trial at this location was conducted in the open-field, whereas the 2017 trial was conducted within a 20’ x 96’ four-season quonset-style high tunnel with no end walls or sidewalls. The 2016 trial included four replications and was arranged in two rows. Each row contained two replications. ‘Karisma’ was used as a nongrafted control and as the scion for the two grafted treatments, which included ‘Maxifort’ and ‘Scarface’ rootstocks. Each plot had seven plants, and in-row plant spacing was 18 inches with rows that were 5 ft apart. One empty space was left between plots. A raised-bed growing system was used, and weeds were suppressed via plastic mulch. Urea was applied at a rate of 100 lb N/acre prior to planting. The 2016 JCPHC trial was planted on 3 June. Harvesting occurred on 25 July, 1, 9, and 24 August, 6, 15, and 26 September, and 13 October.

The 2017 trial was centrally-located within the high tunnel and was immediately adjacent to the field. The trial was arranged in four rows, each representing a single replication. The five grafted treatments included ‘Maxifort’, ‘Meeting’, ‘Scarface’, ‘Sweetie’, and ‘Yaocali’ rootstocks. All plots contained between 4 and 6 plants due to limited plant availability. In-row plant spacing was 18 inches and rows were 4 ft apart. One empty space was left between plots. Weeds were suppressed via woven fabric mulch that was placed over the beds and in the walkways. Alfalfa-based organic fertilizer (Gard’n-Wise Organics Earthalizer 4-1-5) was applied at a rate of 100 lb N/acre prior to planting. The 2017 JCPHC trial was planted on 26 May. Harvesting occurred on 31 July, 10 and 21 August, 1, 11, and 27 September, and 9 and 13 October.

Willow Lake Student Farm

A trial was conducted in 2017 at the Willow Lake Student Farm, located in Riley County, KS (lat. 39.249773N, long. 96.573101W). The soil type at this location was a Eudora silt loam (pH = 7.1). The trial at this location included 3 replications and was conducted within a four season, gothic-arch style high tunnel measuring 30 x 48 ft. The trial consisted of two rows divided into thirds, lengthwise. Each replication was located on 1/3 of both rows and were adjacent to each other. Three rootstock treatments were evaluated: ‘Scarface’, ‘Meeting’ and ‘Yaocali’. Each plot had four plants and in-row plant spacing was 18 inches with 5 ft between rows. Weeds were suppressed via woven fabric mulch. Based on the results of a soil test, no fertilizer was applied to the tunnel prior to planting, which is typical at this farm. The 2017 student farm trial was planted on 8 June. Harvesting occurred on 29 August, 11, 15, and 26 September, and 9, 24, and 26 October.

Butler County On-Farm Trial

A trial was conducted in 2017 at Griggs Bros. Farm, a commercial farm located in Riley County, KS (lat. 37.583321N, long. 96.895867W). The soil at this location was an Irwin silty clay loam (pH = 5.9). The trial was conducted within a 4-season high tunnel (35 x 96 ft). The trial was located on the outer two rows of the high tunnel, and included four replications with two replications in each of the two rows. The four grafted treatments included ‘Meeting’, ‘Scarface’, ‘Sweetie’, and ‘Yaocali’ rootstocks. Each plot contained between four and six plants due to limited plant availability. In-row plant spacing was 12 inches and rows were 4 ft apart. A raised-bed growing system was used, and weeds were suppressed with plastic mulch within the beds and fabric mulch between the beds. Water-soluble calcium nitrate fertilizer (15.5-0-0) was applied weekly at a rate of 1 lb/acre/day once fruit set began, and thereafter through the end of the season (estimated 140 lbs N/acre total). The on-farm trial at this location was planted on 2 May 2017. Harvesting occurred on 31 July, 8 and 20 August, 9 and 23 September, 12 and 31 October, and 14 and 20 November.

Data Collection

To account for potential plant losses and enable the calculation of accurate “per-plant” yield values, the number of live plants were recorded, and vigor was visually rated throughout the growing season. All fruit that was at the breaker stage or beyond was harvested regardless of size, and mature green fruit were also harvested according to commercial standards for size in the region (Hartz et al., 1996; Sanchez et al., 1993). Pepper fruit were graded into marketable and nonmarketable categories based on size, the presence of fruit diseases, blossom end rot, and/or pest damage (Hartz et al., 1996). Marketable and nonmarketable fruit data was combined to create measures of total production. On the day of the final harvest, all fruit larger than 1.5 inches in diameter were harvested and added to the total yield, all such undamaged fruit were included in the marketable yield as well. At the final harvest date in all trials, two centrally-located plants were destructively sampled to determine above and below-ground biomass. Plant height was measured from the soil to the meristem of the longest plant stem. Plant roots were washed prior to drying and all samples were dried at 160°F (71.1°C) for approximately 6 days using a Grieve Industrial Shelf Batch Oven model SC-400 (The Grieve Corporation, Round Lake, Illinois) and weighed.

Data Analysis

All raw data was first converted to a per-plant basis to control for plot size. All data were analyzed in SAS Studio: University Edition (version 9.4; SAS Institute Inc. Cary, NC).

Differences in available trial space resulted in trial-to-trial differences regarding which rootstocks were used. These differences inhibited our ability to run factorial ANOVAs comparing production data across all trials and rootstocks at the same time. Additionally, a One-Way Analysis of Variance (ANOVA) found that significant trial*treatment interactions occurred when the data from all trials were combined. Therefore, all yield data was first standardized for each trial using the z-score standardization procedure. The z-score standardization procedure assumes each data set is normally distributed (RGalleon, 2018) and then standardizes it around a mean of "0" and a standard deviation of "1"(Field, 2005; SPSS, 2016; Bhalla, 2017) without changing the distribution (SPSS, 2016). This allows for the comparison of certain independent variables across multivariate data sets, which have significant differences in mean, range and standard deviation (Field, 2005; SPSS, 2016; Bhalla, 2017; Shaw and Brown, 2017; Ling, 2018). While this approach does not allow for quantitative rootstock-to-rootstock comparisons, it provides substantially more statistical power for comparing an individual rootstock to the nongrafted plants.

After standardization, the data was divided by rootstock, and each rootstock was paired with the nongrafted (control) for every trial that included that rootstock. Factorial ANOVAs were then run to determine main and interaction effects (Table 3.1), Normality and homogeneity of variance were then assessed and post-hoc tests were performed. When assumptions of both normality and variance homogeneity were met, a standard one-way ANOVA and Tukey's post-hoc test were used. Mean values for all the transformed data were back-transformed prior to reporting the results (Manikandan, 2010).

The normal distribution was tested for each sub-set of data prior to other statistical analysis using the Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling Goodness-of-fit tests for the normal distribution. Data sets failing any of these three tests at a 95% confidence interval were determined to have a non-normal distribution. Several of the data sets failed the assumption of a normal distribution. Because transforming the data logarithmically was not effective for normalizing the data, post-hoc tests that were robust for non-normal distributions were used as needed. Because of the two-level nature of our data sets (comparing each rootstock to the nongrafted plants), a non-parametric ANOVA utilizing the Wilcoxon Rank-Sum Test (aka the Mann-Whitney U Test) was used to determine whether values for plants grafted to each rootstock were significantly different from the values for nongrafted plants (Glen, 2016; Noether, 1991; Perezgonzalez, 2012). Due to the relatively small sample size, the exact Wilcoxon Two-Sample Test was used to find *P*-values and determine significance for each test (SAS, 2009) (Table 3.2). For data sets failing to meet variance homogeneity (as determined by Levene’s test for Homogeneity of Variance), a Welch’s ANOVA followed by Dunnett’s test, which is robust in regards to unequal variance (Shingala and Rajyaguru, 2015) was used to compare the total yield of grafted plants with the total yield of the corresponding nongrafted plants (Table 3.2).

As often as was possible, the same test was used across all rootstocks for a given measurement (Table 3.2). However, because of rootstock-to-rootstock discrepancies in normality and variance homogeneity, three different tests were used to compare the total yield (lbs per plant) of grafted to nongrafted plants. Because the total fruit yield of plants grafted with ‘Scarface’ rootstock failed the assumption of homogeneity of variance, a Welch’s ANOVA followed by Dunnett’s test was used to compare the total yield of plants grafted to ‘Scarface’ with the total yield of the corresponding nongrafted plants (Table 3.2). The data for the total fruit yield of plants grafted with ‘Maxifort’, failed tests for normality, and was analyzed using a non-parametric ANOVA and the Wilcoxon Rank-Sum Test. All other varieties (meeting assumptions of both variance homogeneity and normality) were analyzed using a standard ANOVA and a Tukey’s post-hoc test.

Pearson’s correlation analysis was used to observe the relationship between yield data and measurements of plant morphology, including above and below-ground biomass and plant height.

Results

Table 3.1 shows the P-Values of factorial ANOVAs for each of the rootstocks, which were used to determine main and interaction effects. There were significant interactions for the marketable fruit number of ‘Scarface’ and marketable fruit size of ‘Sweetie’. Therefore, data from each trial are presented separately in Table 3.3 for marketable fruit number for ‘Scarface’ and marketable fruit size for ‘Sweetie’. Where there were no significant interactions, combined data are presented in Table 3.3 as main effects. Table 3.4 shows the total yield (marketable + nonmarketable) parameters, and data from each trial are presented separately where significant interactions were observed (Table 3.1). Similarly, Table 3.5 shows the percent marketability of each rootstock and data is presented by trial as needed.

When all grafted and nongrafted data were combined, correlation analysis found strong and statistically significant positive relationships between above-ground biomass and both marketable and total fruit weight and marketable and total fruit number (Table 3.6). Similar, although slightly weaker correlations were found for below-ground biomass. Plant height appeared to have a much weaker, though still significant positive correlation with all measures of yield except for total fruit number ($P = 0.1832$; Table 3.6). Table 3.7 shows the above- and below-ground biomass of grafted and nongrafted plants and trial data is presented separately where significant trial*rootstock interactions occurred (Table 3.2).

Scarface

‘Scarface’ was the only rootstock that was tested in all five trials and its use had a significant effect on marketable fruit weight ($P < 0.01$; Table 3.1). Marketable yield was increased by 32% (% change due to grafting = (grafted plant value / nongrafted plant value) - 1) compared to the nongrafted plants ($P < 0.05$; Table 3.3). There were significant trial*rootstock interactions for total yield ($P < 0.05$; Table 3.1) and the data from the individual trials are presented in Table 3.4. Total yield was significantly improved by grafting onto ‘Scarface’ at the Willow Lake location only ($P < 0.05$; Table 3.4) where the use of ‘Scarface’ rootstock improved the total yield of ‘Karisma’ by 45% as compared to the nongrafted plants (Table 3.4). The use of ‘Scarface’ rootstock did not significantly affect the marketable fruit number (Table 3.3) and had inconsistent effects on total fruit number. In the student farm trial, the use of ‘Scarface’ improved the total fruit number by 23% over the nongrafted plants ($P < 0.05$; Table 3.4), while in the OHREC trial, ‘Karisma’ grafted to ‘Scarface’ produced 20% fewer fruit than nongrafted plants ($P < 0.01$; Table 3.4). All other trials showed no statistically significant difference in total fruit number (Table 3.4). The use of ‘Scarface’ rootstock increased marketable fruit size by 15% ($P < 0.05$; Table 3.3) and total fruit size by 18% ($P < 0.01$; Table 3.4) as compared to the fruit size (lbs/fruit) of corresponding nongrafted plants. Increases in percent fruit marketability were significant for plants grafted to ‘Scarface’ ($P < 0.05$; Table 3.1) as compared to nongrafted plants, and ranged from 9% by weight ($P < 0.01$; Table 3.5) to 12% by number ($P < 0.05$; Table 3.5). The main effects showed that while plants that were grafted with ‘Scarface’ rootstock produced significantly (35%) greater above-ground biomass than nongrafted ‘Karisma’ ($P < 0.05$; Table 3.7), grafting to ‘Scarface’ had no significant effect on below-ground biomass.

Yaocali

‘Yaocali’ was tested in all three trials that were conducted in 2017. While ‘Karisma’ grafted to ‘Yaocali’ rootstock did not show statistically significant improvements in marketable

(*P* = 0.053; Table 3.3) or total fruit yield by weight (*P* = 0.067; Table 3.4) over nongrafted 'Karisma', the effect of rootstock was significant when a factorial ANOVA was conducted ($P < 0.05$; Table 3.1). Similarly, the effect of rootstock was significant for marketable fruit number produced ($P < 0.05$; Table 3.1), though the differences between nongrafted 'Karisma' and plants grafted to 'Yaocali' was not significant (*P* = 0.065; Table 3.3). There were no significant main or interaction effects for fruit size, percent marketability, total fruit number or above or below-ground biomass (Table 3.1) and no significant differences for those measurements was observed when they were compared to nongrafted plants.

Meeting

'Meeting' rootstock was tested in the same three trials as 'Yaocali'. For plants grafted to 'Meeting' rootstock was not a significant main or interaction effect (Table 3.1), and no statistically significant differences between 'Karisma' grafted to 'Meeting' and nongrafted 'Karisma' were observed.

Maxifort

'Maxifort' was tested in three trials (both 2016 trials, and the JCPHC high tunnel trial in 2017). The use of 'Maxifort' as a rootstock for 'Karisma' pepper scion significantly reduced both the marketable and total yield (by 85%) as compared to nongrafted plants ($P < 0.001$; Tables 3.3 and 3.4). Similarly, 'Karisma' grafted to 'Maxifort' produced significantly (69%) fewer marketable and (66% fewer) total fruit than the corresponding nongrafted plants (*P* <

0.001; Tables 3.3 and 3.4). For pepper plants grafted to the tomato rootstock ‘Maxifort’, fruit size was reduced significantly (by 40% for marketable fruit, and by 45% in total) ($P < 0.001$; Tables 3.3 and 3.4). Plants grafted to ‘Maxifort’ displayed a 10% decrease in marketability by weight as compared to nongrafted ‘Karisma’ ($P < 0.05$; Table 3.5). ‘Karisma’ grafted to ‘Maxifort’ had significant interaction effects with trial for percent marketability as measured by fruit number ($P < 0.05$; Table 3.1). In the two trials that were conducted at the JCPHC, plants grafted with ‘Maxifort’ showed significant decreases in percent marketability ($P < 0.05$; Table 3.5) as measured by fruit number when compared to nongrafted ‘Karisma’. However, in the trial at OHREC, this trend was not observed. Biomass production for these plants was also reduced significantly, with ‘Karisma’ grafted to ‘Maxifort’ displaying a 93% reduction ($P < 0.001$; Table 3.7) in above-ground biomass, and a 79% reduction ($P < 0.001$; Table 3.7) in below-ground biomass.

Sweetie

‘Sweetie’, a vigorous heirloom cherry tomato variety was tested as a rootstock in two trials (The JCPHC tunnel trial, and the Griggs Bro’s on-farm trial) during the 2017 growing season. Similar to ‘Maxifort’, the use of ‘Sweetie’ as a rootstock for ‘Karisma’ pepper scion showed significant overall reductions in yield and plant growth. The use of ‘Sweetie’ as a rootstock reduced the marketable yield by 89% ($P < 0.001$; Table 3.3), and the total yield by 78% ($P < 0.001$; Table 3.4). Plants grafted to ‘Sweetie’ also produced 73% fewer marketable fruit than the corresponding nongrafted plants (*P* < 0.001; Table 3.3) and 33% fewer total fruit (*P* < 0.05; Table 3.4). ‘Karisma’ pepper scions grafted to ‘Sweetie’ rootstocks also produced 30% smaller marketable fruit (*P* < 0.01; Table 3.3), and 52% smaller fruit in total (*P* < 0.001) than nongrafted controls. Compared to nongrafted ‘Karisma’, plants grafted to ‘Sweetie’ showed significant decreases in percent marketability as measured by both fruit weight and number (32% and 47% respectively) (*P* < 0.001; Table 3.5). Biomass production followed the same general trend as all other measurements taken, with ‘Karisma’ grafted to ‘Sweetie’ showing an 82% reduction in above-ground biomass (*P* < 0.001; Table 3.7), and a 59% reduction in below-ground biomass as compared to nongrafted controls (*P* < 0.05; Table 3.7).

Discussion

The goals of our study were to identify *Capsicum* rootstocks that may be useful for increasing bell pepper yield in the central U.S. and to determine if *Solanum* rootstocks were compatible with bell pepper scions. Therefore, our statistical analysis was designed to identify the effects of grafting with specific rootstocks and not to compare rootstocks to each other. Some general qualitative conclusions may be drawn regarding the merits of two rootstocks based on their performances in relation to the nongrafted variety. However, no quantitative comparisons can be made since each rootstock was not tested in all the trials.

Grafting Bell Pepper for Increased Productivity

Relatively few yield trials with grafted peppers have been reported and most of the pepper rootstocks that are currently available have been developed primarily for disease resistance and/or abiotic stress tolerance (Leal-Fernández et al., 2013). In our study, plants grafted to ‘Scarface’ rootstock showed significantly improved crop productivity for a variety of yield-related parameters. ‘Karisma’ grafted to ‘Scarface’ displayed 32% higher marketable fruit yield than nongrafted plants (Table 3.4), produced 15% larger marketable fruit, and 18% larger fruit overall. Percent marketability was also improved by 9 to 12%. However, it is important to note that ‘Scarface’ was evaluated in all five of the trials that were conducted, likely giving additional power to the statistical tests we ran on it. ‘Scarface’ was also the only rootstock that significantly increased the above-ground biomass of the scion.

As observed in plants grafted to ‘Scarface’ rootstock, we observed positive correlations across all trials between both above- and below-ground biomass and yield (Table 3.6). While the strongest correlations were for above-ground biomass, these observations are consistent with previous research on the physiological characteristics of high-performing grafted pepper plants (Leal-Fernández et al.,(2013), which include larger root size, the production of extra biomass, a larger stem diameter, and according to Soltan, et al.,(2017), overall “vegetative vigor”.

Further testing with scion varieties besides ‘Karisma’ would be valuable, as scion cultivar can play a role in the benefit of grafting with ‘Maxifort’ in tomato (See ch 2 pg 63-64). However, our data suggests that pepper rootstocks ‘Scarface’, and potentially ‘Yaocali’ are good rootstock choices for growers that wish to improve their bell pepper yields, particularly in high tunnel production systems where few identifiable stressors are present. While ‘Yaocali’, did provide some numerical yield advantages as compared to nongrafted plants, statistically significant effects were inconsistent. In our trials, ‘Meeting’ also provided some numerical yield advantage as compared to nongrafted ‘Karisma’, however none of the advantage ‘Meeting’ conferred was statistically significant in any of our trials. Further research is needed to confirm this trend, but it is possible that ‘Meeting’ may be more beneficial for managing diseases like tobacco mosaic virus, phytophthora root rot and corky root rot for which it has at least some resistance.

Intergeneric Grafting

There are multiple reports of successful intergeneric *Capsicum*/*Solanum* grafting (Albacete et al., 2015; Eltayb et al., 2013; Petran, 2013; Rice, 1891; Rodriquez and Bosland, 2010). However, the literature is not entirely in agreement, with Bletsos and Olympios (2008) reporting outright graft failure, Goldschmidt (2014), and Kawaguchi et al.,(2008) describing “severe” incompatibility and Kawaguchi et al.,(2008) reporting consistent premature death of the grafted plants. In all, very little yield data from field trials has been published in the literature regarding the potential of bell peppers grafted to *Solanum* rootstocks. In our trials, both tomato rootstocks, ‘Maxifort’ and ‘Sweetie’ exhibited symptoms consistent with delayed graft incompatibility when paired with ‘Karisma’ scions. These symptoms included reduced growth, reduced yield, undersized fruit, reduced marketability, and higher in-field mortality. Our observations were both consistent and statistically significant, and are similar to those described in the literature (Kawaguchi et al., 2008; Oda et al., 2005).

The *Solanum* rootstocks that we tested were both considered vigorous. ‘Maxifort’ is reported to increase vegetative biomass for tomato (Masterson et al., 2016) (See Ch 2, pg 61) and also carries a number of resistance genes to soilborne plant pathogens as well as viruses, whereas no viral resistance has been reported for ‘Sweetie’. When grafted with pepper scion, both rootstocks responded in a very similar fashion, suggesting that the presence of differential resistance/tolerance to a virus between the rootstock and scion may not explain the observed delayed incompatibility as suggested by Rosskopf et al., (2013, 2014) for tomato, or by Dogra et al., (2018), Mudge (2013), or Rowhani et al., (2017) for a variety of other horticultural crops.

Visual observations of the graft unions of the pepper on tomato grafts showed a high degree of scion undergrowth (rootstock overgrowth), which is often a symptom of delayed incompatibility, though notable discrepancies in stem diameter (typically scion overgrowth) were frequently observed in the pepper on pepper rootstock grafts as well, while these grafts showed no other symptoms of incompatibility. This demonstrates that this deformity does not necessarily indicate the presence of graft incompatibility as mentioned by Bitters, (1986), and Goldschmidt, (2014). The pepper on tomato grafts also had issues related to in-field mortality, with some trials losing 40-50% of plants grafted to tomato rootstocks throughout the season (compared to about 2 to 5% plant loss for nongrafted plants and plants grafted to pepper rootstocks). This plant loss was often due to a failure at the graft union. These observations are consistent with symptoms of delayed incompatibility described by: Gainza et al.,(2015), Goldschmidt (2014), Kawaguchi et al.,(2008), and Oda et al.,(2005).

Heavy lignification occurs in the mature stems of *Capsicum* (Pandey, 1979), likely more so than in *Solanum* or *Nicotiana*, It may be that this structural difference influences the differences in compatibility we observed in our trials. Lignin accumulation and the formation of callus tissue is a reported plant pathogen resistance mechanism, and may play a role in the development of structural barriers that contribute to the incompatibility of some grafts (Andrews and Marquez, 1993). It is likely that such physiological issues might also contribute to the reduction in scion water potential observed in pepper on tomato grafts by contributing to the malformation of vascular tissue at the graft union (Kawaguchi et al., 2008). The scion undergrowth observed in our trials as well as by others (Gainza et al., 2015; Goldschmidt, 2014; Kawaguchi et al., 2008; Oda et al., 2005) could be a visual symptom of this disorder. Given that lignification in peppers occurs primarily in mature tissues, most of that developmental process would not take place until after the graft healing process had been completed. This might explain the apparent delay in incompatibility we observed when bell pepper was grafted onto tomato rootstocks.

Given that a genetic barrier for *Capsicum*/*Solanum* grafting may not be a limiting factor in the success of such grafts, other avenues for overcoming such incompatibility may exist. Cross-compatible tomato accessions, or hybrids have been identified (Albacete et al., 2015;

Chetelat and Petersen, 2003; MacDonald, 2014; Petran, 2013), which could potentially be used as an interstock. Although this would add expense and difficulty to the production process of such annual plants (Hartmann et al., 2013b), the process is relatively commonplace in grafting *Prunus* species for this purpose (Hartmann et al., 2013b; Gainza et al., 2015; Dogra et al., 2018). While impossible through conventional breeding methods, intergeneric *Capsicum*/*Solanum* hybrids might be developed asexually via the micropropagation of polyploid cells that form at the graft union as described by Fuentes et al., (2014) and such a hybrid could be a viable pepper rootstock if it retained some of the beneficial traits of its tomato parent.

Conclusions

While the genetic component of graft compatibility is well established, compatibility remains difficult to predict (Andrews and Marquez, 1993; Venema et al., 2011). Tomato and pepper are closely-related, more so than tomato and tobacco (Park et al., 2011; Rinaldi et al., 2016; Särkinen et al., 2013), and according to Haberal et al., (2016) intergeneric tomato/tobacco grafts demonstrate a high degree of compatibility. However, in this study intergeneric tomato/pepper grafts were not compatible, and most-likely exhibited delayed graft incompatibility. Given this discrepancy between genetic similarity and compatibility, it appears that the genetic component of compatibility may not be the limiting factor in *Capsicum*/*Solanum* grafting, and that differences in the physiology (Hartmann et al., 2013b) and mature stem anatomy (Andrews and Marquez, 1993; Pandey, 1979) may be more important.

Utilizing grafted bell peppers in low disease-pressure growing systems may be a viable method for improving productivity if the right rootstock is utilized. In our trials, ‘Scarface’ showed potential for improving yield and increasing fruit size. Grafting with other pepper rootstocks like ‘Yaocali’ and ‘Meeting’ may also provide some yield benefit, but such rootstocks may be more useful for addressing problems of disease or abiotic stress rather than for improving yield. For growers that are looking to utilize grafting to increase the productivity of their pepper crop, rootstock selection will be an important factor and our trials suggest that ‘Scarface’ and possibly ‘Yaocali” could be viable candidates for high tunnel production in the Central United States. Vegetative vigor, as measured by biomass production was strongly and positively correlated with yield in our trials. For this reason, the use of highly vigorous *Capsicum* accessions may aid in the development of pepper rootstocks with the potential to increase yield in growing systems with minimal abiotic stress or disease pressure from soilborne pathogens. The development of more rootstocks similar to ‘Scarface’ could accelerate the adoption of pepper grafting in the central United States.

Tables

Table 3.1 - Analysis of variance P-values for Main Effects: Trial and Rootstock as well as effect interactions for both marketable and total fruit yield, percent marketability, and both above and below-ground biomass for a sweet pepper rootstock grafting trial conducted in 2016 and 2017 in five locations in central and eastern Kansas. Data was transformed by trial using the z-score standardization procedure prior to analysis.

Rootstock	Effect	Sample Size (n =)	Marketable Fruit			Total Fruit			% Marketability		Biomass (g)	
			Wt. (lbs/plant)	No. (fruit/plant)	Fruit Size (lbs/fruit)	Wt. (lbs/plant)	No. (fruit/plant)	Fruit Size (lbs/fruit)	By Fruit Weight	By Fruit Number	Above Ground	Below Ground
Scarface	**Trial**	19	0.07	<.05	-	<.05	0.11	-	0.11	0.11	0.19	0.33
	Rootstock		<.01	0.07	<.05	<.01	0.21	<.01	<.05	<.05	<.05	-
	Trial*Rootstock		0.11	<.05	-	<.05	<.05	0.22	0.39	-	0.20	<.05
Yaocali	**Trial**	11	0.06	0.12	<.05	<.05	0.16	<.01	0.39	0.30	0.27	0.17
	Rootstock		<.05	<.05	0.95	<.05	0.08	0.06	0.12	0.07	0.15	0.27
	Trial*Rootstock		0.53	0.24	0.48	0.60	0.68	0.33	0.26	0.07	0.15	-
Meeting	**Trial**	11	0.24	0.24	0.18	0.23	0.56	<.05	0.25	<.05	0.10	0.26
	Rootstock		0.06	0.09	0.25	0.07	0.10	0.16	0.11	0.29	0.13	0.23
	Trial*Rootstock		-	-	-	-	-	-	-	-	-	-
Maxifort	**Trial**	12	0.37	0.31	-	0.18	0.10	-	-	-	0.37	0.12
	Rootstock		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.05	<.01	<.0001	<.0001
	Trial*Rootstock		-	-	-	-	-	-	0.05	<.05	0.19	-
Sweetie	**Trial**	7	0.54	-	<.05	0.35	-	0.07	-	-	0.40	-
	Rootstock		<.001	<.001	<.001	<.001	<.05	<.0001	<.001	<.0001	<.0001	<.05
	Trial*Rootstock		0.32	-	-	-	0.34	-	-	-	0.20	-

The listed P-values were determined using the GLM procedure (SAS Studio: University Edition V 9.4; SAS Institute Inc. Cary, NC). P-values above 0.40 are not shown.

Table 3.2 - determination of appropriate statistical procedures for both marketable and total fruit yield, and percent marketability based on the results of Variance Homogeneity and Normality Tests, for a sweet pepper rootstock grafting trial conducted in 2016 and 2017 in five locations in central and eastern Kansas. Data was transformed by trial using the z-score standardization procedure prior to analysis.

		Pepper Rootstocks			Tomato Rootstocks	
		Scarface	**Yaocali**	**Meeting**	**Maxifort**	**Sweetie**
Marketable Yield	**Wt. (lbs/plant)**	TUKEY	TUKEY	TUKEY *	TUKEY	TUKEY
	No. (fruit/plant)	WILCOX Δ	WILCOX	WILCOX*	WILCOX	WILCOX
	Fruit Size (lbs/fruit)	TUKEY	TUKEY *	TUKEY *	TUKEY	TUKEY
Total Yield	**Wt. (lbs/plant)**	WELCH Δ	TUKEY	WILCOX*	TUKEY	TUKEY
	No. (fruit/plant)	TUKEY Δ	TUKEY *	TUKEY *	TUKEY	TUKEY
	Fruit Size (lbs/fruit)	WILCOX	WILCOX*	WILCOX*	WILCOX	WILCOX
Percent Marketability	**By Fruit Weight**	WILCOX	WILCOX*	WILCOX*	WILCOX	WILCOX
	By Fruit Number	WILCOX	WILCOX*	WILCOX*	WILCOX Δ	WILCOX
Biomass	**Above Ground (g)**	TUKEY	TUKEY *	TUKEY *	TUKEY	TUKEY
	Below Ground (g)	WILCOX Δ	WILCOX*	WILCOX*	WILCOX	WILCOX

Normal Distribution tests with a 95% confidence interval were used and included Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling goodness-of-fit tests for the normal distribution. Levene's Test for Homogeneity of Variance with a 95% confidence interval was used to test for Variance Homogeneity for all metrics of each rootstock, Scarface: Total Yield (lbs/plant) was the only metric to fail this test. Statistical Tests were chosen based on the assumptions met or failed by each data subset. For data with a normal distribution and variance homogeneity, a Standard One-Way ANOVA + Tukey's Post Hoc Test (TUKEY) was used. For data with a non-normal distribution, a Nonparametric ANOVA (Wilcoxon Rank-Sum Test) with an Exact Wilcoxon 2-sample test (WILCOX) was used. For data failing the assumption of variance homogeneity, a Welch's ANOVA + Dunnett's 2-Tailed Post-Hoc Test (WELCH) was used. Where possible, the same test was used across all rootstocks for a given measurement.

The Triangle Symbol (Δ) indicates metrics for which "Trial*Rootstock" was a significant effect (See Table 1). These metrics were divided by trial prior to being analyzed.

* Indicate metrics for which "Rootstock" or “Trial * Rootstock” was not a significant effect (see Table 1). For Metrics where "Rootstock" or “Trial * Rootstock” was not a significant effect. For consistency, tests were still run on these metrics as outlined in the table above, but the results of normality and variance homogeneity tests were not taken into account when determining the appropriate statistical procedures for the metric as values for these tests were assumed not to be significantly different from measurements of corresponding nongrafted plants.

Table 3.3 - Marketable yield and fruit size for a sweet pepper rootstock grafting trial conducted in 2016 and 2017 in five locations in central and eastern Kansas. Data was transformed by trial using the z-score standardization procedure prior to analysis, and back transformed for presentation here.

				Marketable fruit yield (lbs/plant)			**Marketable fruit (no./plant)**			**Avg. mkt. fruit size (lbs/fruit)**		
	Rootstock	**Trial**	**n=**	**Grafted**	**Nongrafted**	***P* < 0.05**	**Grafted**	**Nongrafted**	***P* < 0.05**	**Grafted**	**Nongrafted**	***P* < 0.05**
Capsicum	**Scarface**	**2016 OHREC**	4	7.23	5.48	*	31.2	36.1	0.114	0.29	0.25	*
		2016 JCPHC (Field)	4				25.5	21.0	0.114			
		2017 JCPHC (Tunnel)	4				29.6	24.6	0.200			
		2017 Student Farm	3				28.1	20.7	0.100			
		2017 Griggs Farm	4				12.1	10.4	0.686			
	Yaocali	**2017 JCPHC (Tunnel)**	4	6.44	4.08	0.053	25.8	17.1	0.065	0.25	0.25	0.932
		2017 Student Farm	3									
		2017 Griggs Farm	4									
	Meeting	**2017 JCPHC (Tunnel)**	4	6.87	4.08	0.069	25.7	17.1	0.171	0.27	0.25	0.239
		2017 Student Farm	3									
		2017 Griggs Farm	4									
Solanum	**Maxifort**	**2016 OHREC**	4	1.02	6.88	***	8.7	28.4	***	0.14	0.24	***
		2016 JCPHC (Field)	4									
		2017 JCPHC (Tunnel)	4									
	Sweetie	**2017 JCPHC (Tunnel)**	4	0.47	4.28	***	4.9	18.1	***	0.16	0.23	**
		2017 Griggs Farm	3									

P-values represent significance level for differences between measures of yield for grafted plants and nongrafted controls

* = P <0.05, ** = P < 0.01, *** = P < 0.001

Table 3.4 - Total yield and overall size for a sweet pepper rootstock grafting trial conducted in 2016 and 2017 in five locations in central and eastern Kansas. Data was transformed by trial using the z-score standardization procedure prior to analysis, and back transformed for presentation here.

				Total fruit yield (lbs/plant)			Total fruit (no./plant)			Avg. total fruit size (lbs/fruit)		
	Rootstock	**Trial**	**n=**	**Grafted**	**Nongrafted**	***P* < 0.05**	**Grafted**	**Nongrafted**	***P* < 0.05**	**Grafted**	**Nongrafted**	***P* < 0.05**
Capsicum	**Scarface**	**2016 OHREC**	4	11.19	12.83	0.242	34.9	43.7	**	0.28	0.23	**
		2016 JCPHC (Field)	4	5.85	4.72	0.088	31.1	27.1	0.204			
		2017 JCPHC (Tunnel)	4	7.30	5.89	0.145	33.3	28.8	0.193			
		2017 Student Farm	3	9.24	6.36	*	33.3	27.1	*			
		2017 Griggs Farm	4	3.75	3.19	0.341	13.3	11.7	0.598			
	Yaocali	**2017 JCPHC (Tunnel)**	4	7.07	4.57	0.067	29.4	20.8	0.094	0.25	0.22	0.270
		2017 Student Farm	3									
		2017 Griggs Farm	4									
	Meeting	**2017 JCPHC (Tunnel)**	4	7.46	4.57	0.093	30.4	20.8	0.123	0.25	0.22	0.217
		2017 Student Farm	3									
		2017 Griggs Farm	4									
Solanum	**Maxifort**	**2016 OHREC**	4	1.23	8.00	***	11.4	34.1	***	0.13	0.23	***
		2016 JCPHC (Field)	4									
		2017 JCPHC (Tunnel)	4									
	Sweetie	**2017 JCPHC (Tunnel)**	4	1.04	4.75	***	14.7	22.0	*	0.11	0.23	***
		2017 Griggs Farm	3									

P-values represent significance level for differences between measures of yield for grafted plants and nongrafted controls

* = P <0.05, ** = P < 0.01, *** = P < 0.001

Table 3.5 - Percent marketability by weight and number for a sweet pepper rootstock grafting trial conducted in 2016 and 2017 in five locations in central and eastern Kansas. Data was transformed by trial using the z-score standardization procedure prior to analysis, and back transformed for presentation here.

				Marketable fruit (% by weight)			**Marketable fruit (% by no.)**		
	Rootstock	**Trial**	**n=**	**Grafted**	**Nongrafted**	**$P < 0.05$**	**Grafted**	**Nongrafted**	**$P < 0.05$**
Capsicum	**Scarface**	**2016 OHREC**	4	93.4%	85.6%	**	88.8%	79.3%	*
		2016 JCPHC (Field)	4						
		2017 JCPHC (Tunnel)	4						
		2017 Student Farm	3						
		2017 Griggs Farm	4						
	Yaocali	**2017 JCPHC (Tunnel)**	4	91.1%	87.8%	0.332	88.2%	80.9%	0.365
		2017 Student Farm	3						
		2017 Griggs Farm	4						
	Meeting	**2017 JCPHC (Tunnel)**	4	91.8%	87.8%	0.151	85.1%	80.9%	0.439
		2017 Student Farm	3						
		2017 Griggs Farm	4						
Solanum	**Maxifort**	**2016 OHREC**	4	75.1%	83.0%	*	84.7%	82.9%	0.886
		2016 JCPHC (Field)	4				32.6%	77.3%	*
		2017 JCPHC (Tunnel)	4				61.9%	85.5%	*
	Sweetie	**2017 JCPHC (Tunnel)**	4	58.9%	87.4%	***	45.0%	84.5%	***
		2017 Griggs Farm	3						

P-values represent significance level for differences between measures of percent marketability for grafted plants and nongrafted controls

* = P <0.05,** = P < 0.01, *** = P < 0.001

Table 3.6 - Pearson Correlation Coefficients for relationship between yield and plant morphological characteristics measured by plant height (ground level to the longest vertical meristem) and dry biomass for a sweet pepper rootstock grafting trial conducted in 2016 and 2017 in five locations in central and eastern Kansas (N = 60).

		Above-ground biomass (g)	**Below-ground biomass (g)**	**Plant height (cm)**
Marketable Fruit	**Wt. (lbs/plant)**	0.87	0.59	0.40
	***P*-value**	***	***	***
	No. (fruit/plant)	0.75	0.60	0.30
	***P*-value**	***	***	**
Total Fruit	**Wt. (lbs/plant)**	0.88	0.63	0.38
	***P*-value**	***	***	***
	No. (fruit/plant)	0.70	0.59	0.15
	***P*-value**	***	***	0.1832

P-values represent significance level for correlation * = P <0.05,** = P < 0.01, *** = P < 0.001

Table 3.7 - Above and Below-Ground Dry Biomass (g) for a sweet pepper rootstock grafting trial conducted in 2016 and 2017 in five locations in central and eastern Kansas. Data was transformed by trial using the z-score standardization procedure prior to analysis, and results were back-transformed for presentation.

Rootstock	**Location**	**n=**	**Below-ground biomass (g)**			**Above-ground biomass (g)**		
			Grafted	**Nongrafted**	***P*-value**	**Grafted**	**Nongrafted**	***P*-value**
Scarface	**Griggs**	4	9.27	6.42	0.49	186.2	138.0	*
	JCPHC Field	4	23.98	22.49	0.89			
	JCPHC Tunnel	4	8.89	8.38	0.31			
	OHREC	4	19.13	25.25	0.11			
	Student Farm	3	13.78	9.61	0.20			
Yaocali		11	13.78	7.49	0.40	146.9	100.1	0.26
Meeting		11	14.43	7.49	0.37	161.7	100.1	0.16
Maxifort		12	3.85	18.69	***	10.3	158.2	***
Sweetie		7	2.91	7.14	*	16.3	91.0	***

P-values represent significance level for differences between measures of percent marketability for grafted plants and nongrafted controls * = P <0.05,** = P < 0.01, *** = P < 0.001

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