

GRAFTING TO INCREASE HIGH TUNNEL TOMATO PRODUCTIVITY IN THE
CENTRAL UNITED STATES

by

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Abstract

As populations of cities continue to increase, communities in the United States are implementing urban food systems including locally-cultivated produce. Urban and peri-urban farmers apply intensive production systems, including high tunnels, to better utilize limited space. Grafting tomato with vigorous rootstocks provides the potential for higher yields. Our first objective was to identify tomato rootstocks that improve productivity in high tunnel environments with no soilborne diseases in the Central U.S. Eight replicated high tunnel trials were conducted at four sites in northeastern Kansas in 2013 and 2014. We selected 'BHN 589' scion for all sites and evaluated seven rootstocks. Grafting with 'Maxifort', 'Multifort', 'Arnold', 'DRO 131', and 'Colosus' rootstocks resulted in significant increases in total fruit yield, which ranged from 40% to 73% when compared to nongrafted plants ($P < 0.05$). No significant increases in yield were observed for 'RT-1028' and 'RST-04-106' rootstocks. Our results suggested that tomato growers that utilize high tunnels should be strategic when selecting rootstocks. Our second objective was to develop simple propagation techniques that yield high quality grafted transplants for small-batch propagators. Formation of adventitious roots (AR) from the scion can result in poor quality plants and loss of rootstock function/benefit. Greenhouse studies were designed to investigate how leaf removal (LR) affects AR formation and plant growth post-grafting. We applied three treatments, 0% LR, 50% LR, and 90% LR, to the 'BHN 589' scion and then grafted them onto 'Maxifort' rootstock. The experiment included 4 replicated blocks and was conducted in three different healing chambers. Our results indicate that both 50% and 90% LR significantly decreased AR formation in the low humidity chamber, but only 90% LR reduced AR formation in the chambers with high humidity ($P < 0.05$). We measured plant growth 24 to 52 days post-grafting to understand how leaf removal affects transplant quality, growth,

and development. Plants with 90% LR had significant growth reduction at day 24 but at day 52, only had reduced stem diameter and height compared to 0% LR. Total flower count was the same for all treatments. Leaf removal during grafting may be a viable method for propagating high quality, grafted transplants.

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Dedication

To good family, good friends, and good neighbors
and my faithful companion Lili.

Chapter 1 - Literature Review

Importance of Local Vegetable Production in the United States

As our population continues to increase, there is growing concern about how to sustainably feed the people without compromising environmental sustainability. About half of the world's population lives in urban settings (United Nations, 2014), which are limited in resources for agriculture such as land and water (Foley et al., 2011; Smit et al., 2001). Due to this growing population density, the concern for global food security – the need for equitable production and distribution of nutritious food - is also rising (Foley et al., 2011; Smit et al., 2001). With 81% of inhabitants living in cities, North America is among the highest urbanized area in the world with the United States contributing approximately 23 million people to its urban population. (United Nations, 2014).

One approach to addressing food security is to create more localized urban food systems where food production and distribution as well as recycling of agricultural resources occur within urban and peri-urban areas (Eigenbrod and Gruda, 2015; Smit et al., 2001). Local food is often referred to when an agricultural product is produced in close vicinity to its market. Although, the market distance can be interpreted in different distances, often, local food production is thought to be within a 100 miles of its market. One aspect of this plan is to grow more food in the urban and the surrounding peri-urban areas (Eigenbrod and Gruda, 2015; Smit et al., 2001). Smit et al. (2001) define urban agriculture as “... *an industry that produces, processes, and markets food, fuel, and other outputs, largely in response to the daily demand of consumers within a town, city, or metropolis, on many types of privately and publicly held land and water bodies found throughout intra-urban and peri-urban areas.*” Peri-urban agriculture

describes food production on the outskirts of the urban core, often with larger-acreage land available for increased production of commodities especially those requiring more space such as animals and grain crops (Smit et al., 2001). Growers with limited growing space like urban growers or greenhouse or high tunnel growers, practice intensive agricultural production methods such as increased plant density within an area, succession planting and intercropping, reduced fallow time, and reuse of water and waste resources (Smit et al., 2001). But even with limited space, well-managed, intensive agriculture systems can often produce more food than traditional agriculture systems within the same space (Smit et al., 2001). Both commercial and noncommercial types of urban agriculture are working together to supply the community with healthy food, educate consumers on sustainable food systems, and protect the environment (Eigenbrod and Gruda, 2015; Smit et al., 2001; Hodgson et al. 2011; Rogus and Dimitri, 2014).

Research indicates that urban agriculture results in several environmental, economic, and social benefits (Eigenbrod and Gruda, 2015; Hodgson et al., 2011; Smit et al., 2001). One social improvement is the increase in access, especially for low-income consumers, and consumption of fresh fruits and vegetables (Eigenbrod and Gruda, 2015; Smit et al., 2001), which helps prevent diseases and maintain healthy weight (U.S. Dept. Health and Human Serv., 2015). Backyard gardens, community gardens, school gardens, food pantry gardens and other institutional gardens supply fresh fruits and vegetables to many urban dwellers (Eigenbrod and Gruda, 2015; Hodgson et al., 2011; Smit et al., 2001). Other people are choosing to purchase local and sustainable produce in part because they desire to be informed about their food origins and journey to their plate (Hunt, 2006). This movement is resulting in new markets that are ultimately reinventing the way that fresh produce is being grown and distributed. These investments in local production and

development of local markets create jobs and potential revenue for economic development in urban areas (Eigenbrod and Gruda, 2015; Smit et al., 2001)

In our current food system, most of our fruits and vegetables are grown in California, Florida, and internationally, where ideal climates and land space offer the ability for large-scale, production (Rogus and Dimitri, 2014). These products are then shipped through complex distribution chains that utilize wholesale markets. Although the United States has a large urban population, it is an extremely large country in area and only 3% of the its total land is used for urban space; thus, allocating land for food production in the best possible climates is more obtainable in the U.S. than other countries (Rogus and Dimitri, 2014). However, the method of large commercial farming systems that are a long distance from the market(s) is environmentally unsustainable for many reasons including the reallocation and consumption of resources, especially fossil fuels from transportation and other agricultural activities (Eigenbrod and Gruda, 2015; Smit et al., 2001). Therefore, with such environmentally degrading activities we are witnessing evidence of global climate change, which ironically creates new challenges in agricultural production.

An urban food system localizes the environmental footprint of agriculture, allowing growers and consumers to better balance inputs and outputs and recycle resources and energy within their food system (Eigenbrod and Gruda, 2015; Smit et al., 2001). Vegetables are ideal commodities for urban agriculture because their production systems can be very profitable within intensive cultivation, and their short shelf life makes them an ideal candidate for shorter distribution to markets (Eigenbrod and Gruda, 2015). It could help reduce waste of vegetables by extending shelf-life, distributing to those in need, and having a system that recycles waste back into the food system (Smit et al., 2001).

The urbanization of agriculture has a number of benefits, but also poses a number of challenges. Perhaps the most significant challenge among these is that urban produce farmers are looking to increase the level of intensity at which they are growing in order to compete with higher land, water, and labor costs (Hodgen et al., 2011; Rogus and Dimitri, 2014; Smit et al., 2001). One limiting factor in urban agriculture is available growing space due to physical availability as well as the difficult accessibility associated with higher land value in urban areas (Eigenbrod and Gruda, 2015; Martellozzo et al., 2014; Smit et al., 2001). In a study by Martellozzo et al. (2014), the authors calculated the percentage of urban agriculture needed (PAN) to fulfill the vegetable demand for an individual country and reported that the United States only needs less than 10% of urban land devoted to agriculture to supply enough vegetables for its inhabitants. Although, it is not guaranteed that enough of the urban land is suitable for production, especially within highly populated metropolitan areas (Martellozzo et al., 2014). However, smaller- and medium-sized cities may have greater capacity and land area to provide enough vegetables to their urban dwellers as well as additional produce for larger cities (Martellozzo et al., 2014). The World Urbanization Prospects report estimates that about half of urban inhabitants live in cities with a population less than 500,000 (United Nations, 2014). Furthermore, incorporating more peri-urban farms into localized food systems can help create an overall more sustainable system with less negative environmental impact (Eigenbrod and Gruda, 2015; Martellozzo et al., 2014).

As our world population increases and threatens our food security, it also continues to threaten the fragility of our environmental ecosystems (Foley et al., 2011). Foley et al. (2011) notes in his report, "Solutions to a Cultivating Planet", "*to achieve global food security and environmental sustainability, agricultural systems must be transformed to address both*

challenges.” Furthermore, Foley et al. (2011) adds that a significant portion of energy should be focused on increasing production use of the land that we have already drastically impacted before conquering other sensitive areas. However, the intensification of the land must be approached with sustainable methods to further protect and improve our environment (Foley et al., 2011). In the report, Foley et al. (2011) concludes that: (1) solutions should focus on critical biophysical and economic ‘leverage points’ in agricultural systems, (2) new practices must also increase the resilience of the food system, (3) agricultural activities have many costs and benefits, but methods of evaluating the trade-offs are still poorly developed, and (4) the search for agricultural solutions should remain technology- neutral.

Many small- and mid-sized cities are found in the United States, including the Central U.S. region. In 2007, Kansas City, MO ranked 29th in a list of 50 U.S. cities with the highest population. Furthermore it reported to have 15,529 urban and peri-urban agriculture farms with an approximate 3,607,000 acres in farmland, which was the most area reported by all 50 cities (Rogus and Dimitri, 2014). Rogus and Dimitri (2014) found that clustering of small and medium sized farms mainly formed in urban and peri-urban areas with low land value and less dense population. Moving vegetable production close to the cities and rethinking the way we distribute food will also require vegetable growers to adjust the way we produce food.

If we look at other areas of the world that have been feeding large densely-populated areas, such as Asia and Europe, we see that they utilize intensified protected agriculture systems and grafting with resistant/tolerant rootstocks to decrease reliance on chemicals, particularly for cucurbit and solanaceous crops (Lee 1994). Both of these technologies help farmers increase food production in smaller spaces. As the United States vegetable production in and around cities intensifies, more growers may need to adopt practices such as high tunnels and grafting to help

increase food production in confined growing spaces.

High Tunnel Production

In response to the increase of consumer-driven local markets, produce growers in the U.S. and around the world are constructing high tunnels with the hope that protecting crops adverse conditions will increase yield and quality (Carey et al., 2009; Lamont, 2009). A high tunnel is an unheated structure, typically with a single layer of greenhouse grade plastic covering and passive ventilation through adjustable sides and end walls and with crops growing directly in the soil (Wells and Loy, 1993). The simplistic design of high tunnels, which often requires less investment than permanent greenhouses, offers growers an affordable technology for intensive crop production (Wells and Loy, 1993). These structures extend the growing season of many high value crops and potentially increase growers' profits depending on production management, marketability, and environmental factors (Blomgren and Frisch, 2007; Conner et al., 2010; Everhart et al., 2009; Galinato and Miles, 2013; Sydorovych et al., 2013; Waterer, 2003; Wells and Loy, 1993). A plasticulture system, including high tunnels, drip tape, and plastic mulch is especially advantageous for growers in areas with high population density, limited land and water resources, and climatic issues (Wittwer, 1993), which describes most urban areas (Foley et al., 2011; Smit et al., 2001).

Although high tunnel research in the United States began in the 1950's, plastic-covered production of high-value fruit and vegetables, whether a high tunnel or greenhouse, spread more rapidly in Asia and Mediterranean countries (Wittwer, 1993). Wittwer (1993) reported that China's approximate 62,000 hectares of plastic-covered production was cultivated for fruit and vegetables, while the United States' plastic-protected 3,500 hectares mostly produced potted

plants and flowers. However, since that time, U.S. researchers' and growers' interest in high tunnel systems for fruit and vegetables has been continually increasing especially for small-acreage growers selling to local markets (Carey et al., 2009; Lamont, 2009; Wells and Loy, 1993). Carey et al. (2009) reported that a majority of the states are active in high tunnel production and research for the season extension and quality improvement of fruit, vegetable, and/or cut flower crops.

More recent data indicates the importance of these structures in the Central U.S., often called the Great Plains growing region (Kansas, Missouri, Iowa, Nebraska), but could also include Oklahoma, eastern Colorado. Knewton et al. (2010a) reported that a majority of Great Plains high tunnel growers use high tunnels for fruit or vegetable production with tomato being the most commonly-grown crop. Furthermore, at the 2014 Great Plains Growers Conference in St. Joseph, MO, we surveyed vegetable, fruit, and flower growers (n=265), who reported that 42% currently use high tunnels, 23% currently use high tunnels and want to add more, and 18% would like to incorporate them into their operations (C.L. Rivard, unpublished data). Taken together, 83% of growers surveyed are either using high tunnels or would like to add them to their farm businesses. The majority of the survey participants were rural farmers (71%), but 7% of respondents identified themselves as commercial urban farmers (C.L. Rivard, unpublished data). High tunnels could be especially advantageous for urban farmers looking to produce high quality produce and supply a quantity of vegetables year-round in a limited space (Lamont, 2013). They can be constructed as temporary structures, which may forgo additional property taxes (Blomgren and Frisch, 2007; Everhart et al., 2009; Wells and Loy, 1993) and also benefit growers leasing plots of land.

The High Tunnel Initiative, supported by the Nature Resource Conservation Service (NRCS) Environmental Quality Incentives Program (EQIP), helps fund eligible growers in all states in purchasing high tunnels for their production of agriculture commodities (U.S. Dept. Agr., n.d.). The NRCS recognizes that high tunnel systems benefit growers with crop and soil protection, consumers with high quality local vegetables, and the environment with reduced transportation energy consumption (U.S. Dept. Agr., n.d.). Cleveland is an example city that used the High Tunnel Initiative to begin a pilot project in 2012 to help urban growers extend the growing season, especially in areas with limited access to fresh produce (U.S. Dept. Agr., 2014). The first year all 23 applicants were awarded the 90% payment rate and by 2014, applications nearly doubled with over 43 growers applying to be involved with the program, which highlights the growing interest in high tunnel production for urban growers (U.S. Dept. Agr., 2014).

Other cities have also launched high tunnel projects to help increase food production in the urban core. In Philadelphia, Penn State University and county extension offices are partnering with local schools, parks, non-profits, and other community organizations to erect high tunnels to increase year around fresh produce for areas with limited access to healthy food (Lamont, 2013). Beyond just production, the project is centered on teaching and engaging the local community in growing food and healthy eating (Lamont, 2013). As the use of high tunnels continues to rise in the central U.S. region, it is apparent that fruit and vegetable growers will need more regional information to facilitate the adoption of protected cultivation, especially to facilitate the rise of urban agriculture.

Benefits of Growing in High Tunnels

Vegetable Growers in the United States are adopting high tunnel operations as a result of numerous benefits that they provide to crops. High tunnels can protect crops against abiotic and

biotic stresses such as rain, hail, wind, low temperatures, wildlife, insects, weeds, and disease (Blomgren and Frisch, 2007; Everhart et al., 2009; Wells and Loy, 1993). The utilization of high tunnels allows growers to better control the environment compared to the open-field, although still with less control than a permanent, heated greenhouse (Blomgren and Frisch, 2007; Everhart et al., 2009; Lamont, 2009; Wells and Loy, 1993). Growers particularly use high tunnels for extending crop production early into spring, late into fall, and in some climates year-round (Wells and Loy, 1993). O'Connell et al. (2012) reported that high tunnel tomato harvests began approximately 3 weeks earlier than field harvests in North Carolina. A study in New Jersey showed that during spring, high tunnels can increase nighttime air and soil temperatures by 0.9° C and 6.7° C, respectively, compared to the open-field (Both et al., 2007). Moreover, the study utilized an energy curtain, similar to a row cover, within the tunnel that was able to increase air and soil temperatures 2.3° C and 7.2° C, respectively, compared to open-field temperatures (Both et al., 2007). Research by Hunter et al. (2012) in northern Utah also indicates that the addition of row covers and simple heat sources such as temporary heaters, heating lamps, and/or soil heating coils inside the tunnels further protect crops from frost so as to facilitate earlier planting dates. Additional accumulated degrees may allow for better early growth and oftentimes, earlier maturation of plants (Both et al., 2007; Hunter et al., 2012; O'Connell et al., 2012; Rogers and Wszelaki, 2012; Waterer, 2003). One of the major benefits of early growth and maturation is that out-of-season harvests can lead to better market prices, particularly in direct markets (Blomgren and Frisch, 2007; Galinato and Miles, 2013; Hunter et al., 2012; Sydorovych et al., 2013; Waterer, 2003).

Growing in high tunnels can also yield higher quality crop by physically protecting the commodity from biotic and abiotic stressors: wildlife, disease, hail, rain, temperature, radiation,

wind, etc. (Blomgren and Frisch, 2007; Everhart et al., 2009). Studies have shown that high tunnels can increase total marketable yield of tomatoes compared to open-field production (Galinato and Miles, 2013; O'Connell et al., 2012; Rogers and Wszelaki, 2012). With the exclusion of precipitation and use of drip irrigation, growers can keep foliage dry and regulate soil moisture. Thus, the ability to regulate water can improve plant and fruit quality by reducing the incidence of foliar diseases and fruit defects associated with water fluctuations such as cracking and blossom end rot; this is particularly well-suited for certified organic production (O'Connell et al., 2012; Rogers and Wszelaki, 2012).

Tomato is the most commonly-cultivated crop in high tunnels in the world (Lamont, 2009), United States (Carey et al., 2009), and more specifically, the Central U.S. (Knewton et al., 2010a). However, profitability depends on many variables such as climate, market price, operations, and management (Blomgren and Frisch, 2007; Conner et al., 2010; Everhart et al., 2009; Galinato and Miles, 2013; Sydorovych et al., 2013; Waterer, 2003). High tunnels may be especially appealing to protect heirloom tomatoes, which are often susceptible to cracking and bruising and usually are direct marketed with premium prices (Jordan, 2007; O'Connell et al., 2012; Rogers and Wszelaki, 2012; Sydorovych et al., 2013). With the increased interest in local food, small acreage and/or urban farms are supplying more heirloom tomatoes at farmers' markets, restaurants and high-end supermarkets (Jordan, 2007). High tunnel growers in urban and peri-urban areas may benefit from the advantage of extending their local supply of premium priced high quality heirloom tomatoes, which are often unobtainable through a more distant shipping system (Rogers and Wszelaki, 2012).

Challenges of Growing in High Tunnels

High tunnels can pose various challenges like cost, labor, pests, planting dates, soil and nutrient management, and limitations associated with confined growing space (Blomgren and Frisch, 2007; Everhart et al., 2009; Galinato and Miles, 2013; Knewtson et al., 2010a). Although less expensive than a greenhouse, high tunnels require a significant initial investment for the structure and maintenance adds costs in particular years. The metal structure is estimated to last 30 years, but wooden baseboards and end-walls need to be replaced after approximately 10 years and the polyethylene film covering should be replaced about every 4 years (Blomgren and Frisch, 2007; Everhart et al., 2009; Sydorovych et al., 2013); poly-film may also be replaced sporadically if it damaging storms with high winds and large hail occur, which is not uncommon in the Central U.S. There are several economic analyses of high tunnel enterprise budgets. Based on high tunnel case studies and economic reports, a high tunnel will typically pay for itself in one to five years (Blomgren and Frisch, 2007; Conner et al., 2010; Sydorovych et al., 2013; Waterer, 2003). In addition to material costs, high tunnels require more labor for maintenance, operation, and extended harvesting (Conner et al., 2010; Galinato and Miles, 2013; Knewtson et al., 2010a; Sydorovych et al., 2013). Many tasks can be performed in high tunnels virtually year-around and/or in weather conditions that may normally limit fieldwork in the open-field. Galinato and Miles (2013) estimated that a Washington tomato grower's total production costs were eight times greater in high tunnels when compared to field production and labor was the major contributing factor in cost. However, despite the increase in variable costs, tomato crops grown in the high tunnel system were three times more profitable than an open-field grown crop (Galinato and Miles, 2013).

Maintaining proper ventilation in high tunnels is very important (Wells and Loy, 1993), especially to avoid heat stress and humidity (Blomgren and Frisch, 2007; Everhart et al., 2009). In eastern Tennessee, Rogers and Wszelaki (2012) recorded temperatures up to 125° F inside high tunnels and speculated such extreme heat stress most likely caused low yields; although, it was not stated if the high tunnels were properly vented. In climates with high temperatures, growers may want to consider supplemental ventilation and cooling systems (Rogers and Wszelaki, 2012), as well as shade cloth for areas with intense solar radiation (Blomgren and Frisch, 2007; Everhart et al., 2009). Ventilation is also important to decrease humidity, which can also help to manage foliar diseases (Blomgren and Frisch, 2007; Everhart et al., 2009).

Because of high tunnels' intensively-cultivated spaces and warmer microclimate compared to open-field production, they can potentially harbor weeds, insects, and pathogens in the soil and/or structure (Blomgren and Frisch, 2007; Everhart et al., 2009). Tomato growers have reported diseases such as leaf mold (*Fulvia fulva*), powdery mildew (*Podosphaera xanthii*), bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*), and verticillium wilt (*Verticillium dahliae*) (Blomgren and Frisch, 2007). For foliar diseases, proper management of the high tunnel can lead to decreases in the incidence or severity of the epidemic (O'Connell et al., 2012; Rogers and Wszelaki, 2012). However, the management of verticillium wilt and other soilborne diseases has been identified as a major issue in high tunnel production (Blomgren and Frisch, 2007; Everhart et al., 2009). Among other sanitation practices, growers use crop rotation strategies to avoid persistent pests and diseases (Blomgren and Frisch, 2007) but with a high tunnel's limited space, this can be a challenge (Everhart et al., 2009). Soil fumigants or chemigation with fungicides could be implemented to reduce soilborne diseases, but such as the fumigant methyl bromide is being phased-out of production use, many are also highly regulated

due to environmental concerns; growers and researchers are searching for more sustainable pest-management strategies (King et al., 2008; Louws et al., 2010). In addition, the practical implementation of soil fumigants in small high tunnels is difficult and does not coincide with organic or reduced pesticide production practices often used by small-scale farmers that directly market to consumers.

High tunnels must also be managed properly in order to successfully manage soil quality, especially soil salinity (Blomgren and Frisch, 2007; Everhart et al., 2009; Knewton et al., 2010b, 2012). With the increase in productivity, crops grown inside high tunnels benefit from additional nutrient and organic matter inputs (Blomgren and Frisch, 2007; Montri and Biernbaum, 2009). However, large applications of fertilizers and composts in combination with drip irrigation can cause a build-up of salts that would otherwise would be leached out with rains in the open field (Blomgren and Frisch, 2007; Knewton et al., 2010b; Montri and Biernbaum, 2009; Sánchez, 2015). Tomato is considered moderately sensitive to salinity and productivity can be reduced in soils with an EC of 3.5 dS/m (Blomgren and Frisch, 2007; Everhart et al., 2009). However soil salinity in high tunnel soils depends on many factors such as soil management and inputs, soil type, and irrigation quantity and quality (Blomgren and Frisch, 2007; Everhart et al., 2009; Knewton et al., 2010b, 2012; Montri and Biernbaum, 2009; Sánchez, 2015). Studies at Penn State (Sánchez, 2015) showed high tunnel soil salinity levels ranging from 0.37 to 9.38 dS/m. From a study at K-State Olathe Horticulture Research and Extension Center (OHREC), Knewton et al. (2012) reported that after eight years of production in both organic and conventional systems, average soil salinity levels in high tunnel, 0.16 dS/m (conventional) and 0.065 dS/m (organic), did increase compared to open-field, 0.30 dS/m (conventional) and 0.059 dS/m (organic), but not at damaging levels. In addition, high tunnels and fields with organic soil

amendments showed an increase in salinity compared to conventionally-managed systems (Knewton et al., 2012). Many growers, particularly organic farmers, use composted animal manure for soil fertility management, but over application can lead to excess soil salinity (Blomgren and Frisch, 2007; Everhart et al., 2009; Montri and Biernbaum, 2009; Sánchez, 2015).

Depending on water quality and quantity, irrigation source and frequency can be a contributing factor in soil salinity. Irrigation water that is high in dissolved mineral salts can cause a build-up in soil salinity if not managed correctly. These salts are derived from eroding rock in historically marine areas, agriculture run-off and other drainage, and saline water tables (Hanson et al., 2006). Areas with limited rainfall often experience soil salinity (Hanson et al., 2006). High tunnels, which mimic a rainless, dry environment, rely on irrigation water and using well, rural, or municipal water high in salts can cause soil salinity peripheral to drip irrigation (Hanson et al., 2006).

Soil salinity can increase inside tunnels but the rate and severity depends on management practices (Blomgren and Frisch, 2007; Everhart et al., 2009; Knewton et al., 2010b, 2012; Sánchez, 2015). Some growers collapse or move the structure, or remove the plastic, in order to expose the soil to the rain and the open environment for a period of time (Blomgren and Frisch, 2007). If irrigation water and soil permeability is not an issue, than the high tunnel can be flooded to help leach any salts (Blomgren and Frisch, 2007; Everhart et al., 2009; Sánchez, 2015). Another suggestion is to physically remove the top layer of soil that is high in salts and replace with new soil (Blomgren and Frisch, 2007), but this method is highly laborious and often unfeasible. Increasing organic matter with cover crop rotations can also be a valuable method for increasing soil health (Montri and Biernbaum, 2009). These techniques, however, require labor

and other limitations such as cost, space, wear on plastic or structure, and/or timing of crop production. Particularly in an urban setting, moving a structure or leaving a space uncultivated may not be economically feasible for a grower due to the intensive nature of urban production and confined production spaces.

High tunnels require initial investment and intensive crop and soil management, but they can help growers achieve a higher profit in a small area, especially with tomato crops. The protection and control of environment helps increase quantity and quality of produce, but the potential for soil, pest, and disease issues must be monitored and managed to assure consistent and profitable production.

Grafting for High Tunnel Production

Historically, the use of grafted vegetables worldwide has been associated with intensive production systems in protected environments in order to help manage biotic and abiotic stressors (Kubota et al., 2008; Lee et al., 1994, 2010; Oda, 2002). Growers began using vegetable grafting in Japan and Korea in the 1920's as a management strategy for soilborne pathogen *Fusarium* for melons (*Citrullus lanatus*) (Lee, 1994 and 1998; Kubota et al., 2008). It gained popularity with the rise of continuous cropping systems under protected cultivation and became common for many cucurbit and solanaceous species during the 20th century (Kubota et al., 2008; Lee et al., 1994, 2010). Researchers began exploring and identifying other advantages grafting could offer vegetables especially in alleviation of stressful environments associated with intensive agriculture and increase of productivity (Kubota et al., 2008; Lee et al., 1994, 2010; Oda, 2002). As stated in previous section, vegetables produced in successive, high density plantings and off-season production in high tunnels can experience these stresses including: high

humidity, decreased light, increased demand of fertilization, soil-borne pathogens, and extreme temperatures (Lee et al., 2010). Without alleviation from less than desired environment, intensive agriculture can lead to soilborne and foliage diseases, physiological disorders, and decrease in plant and fruit quality (Lee et al., 2010).

By 1992, 59% of Japanese and 81% of Korean cucumber, melons, tomatoes and eggplant greenhouse and field production used grafted plants which utilized nearly 1 billion grafted vegetable plants annually in Japan and Korea combined (Lee, 1994). The technology then spread to Europe (Oda, 2002), and with more efficient propagation technology and seed availability, researchers and growers began adopting the practice around the world including recently in the United States (Kubota et al., 2008).

In the early 2000's a survey from University of Arizona reported that over 40 million vegetable seedlings were grafted in North American, which were mainly utilized in hydroponic tomato greenhouses in Mexico and Canada (Kubota et al., 2008). The use of grafted vegetables is uncommon in the United States because of its vast available agricultural acreage compared to other countries (Lee, 1994) but also because of the unfamiliarity of its technology, cost, and application (King, et al., 2008; Kubota et al., 2008; Lewis et al., 2014). However, in the last decade, research in tomato grafting has been steadily increasing in the United States for both open-field and high tunnel production systems, particularly as the use of intensive cropping systems has increased in production areas with limited growing space. Industry and university researchers along with producers began combining their efforts to identify the efficacy of grafting as a way to reduce soilborne pathogens and decrease reliance on the soil fumigant methyl bromide for U.S. farmers (King et al., 2008; Kubota et al., 2008; Lewis et al., 2014; Louws et al., 2010). As vegetable grafting is mostly used for protected cultivation worldwide

(Lee et al., 2010), it is a logical assumption that as high tunnel production increases in the United States, so will the use of grafted tomato plants. Currently, small-scale growers in the U.S. are showing most interest to apply grafting technology into their production (Lewis et al., 2014)

Benefits of Grafting

Continued research and breeding with grafted vegetable plants has determined the utility of this technology at managing various abiotic and biotic stressors; many of which are catalyzed by the intensified soil cultivation and other management practices that are common in high tunnel systems. Growers and researchers are working to identify rootstocks that tolerate regional issues influencing plant health and productivity (Kubota et al., 2008; Louws et al., 2010).

Many of the pathogens that cause soilborne and/or root diseases can survive without a host for several years making crop rotation challenging and less effective, especially for intensively-cultivated production systems and urban growing spaces (King et al., 2008). Recent reports from the United States have indicated that grafting tomatoes with resistant rootstocks can help manage many devastating soilborne diseases: fusarium wilt (*Fusarium oxysporum* f.sp. *lycopersici*) (Rivard and Louws, 2008), bacterial wilt (*Ralstonia solanacearum*) (McAvoy et al., 2012; Rivard et al., 2012), southern blight (*Sclerotium rolfsii*) (Rivard et al., 2010a), and root-knot nematodes (*Meloidogyne* spp.) (Rivard et al., 2010a; Barrett et al., 2012c). Grafting is an especially important disease management tool for organic growers or those wanting to produce tomato varieties like heirlooms that are susceptible to soilborne pathogens (Rivard and Louws, 2008; Rivard et al., 2010a; Barrett et al., 2012c).

Numerous private and public breeding programs have identified and/or bred rootstock-specific tomato cultivars that carry a number of major resistance genes to soilborne and virus diseases; among these, many have released interspecific hybrid rootstock cultivars (Guan et al.,

2012; Louws et al., 2010). Although several of these rootstocks confer higher vigor when grafted onto scion cultivars as compared to their nongrafted counterparts (Leonardi and Giuffrida, 2006; Masterson, 2013;), it isn't clear what specific traits contribute to this added vigor and how they may perform in different environments (Guan et al., 2012; Kubota et al., 2010; Schwarz et al., 2010). In 2010, Schwarz et al. (2010) identified this issue as a major barrier among breeding programs, as it's not clear what physiological characteristics confer this trait. Schwarz et al. (2010) noted that because we have limited knowledge of abiotic stress resistance in rootstocks genetically, it is difficult to breed vigorous rootstock for specific or broad abiotic stress management. Thus, breeders must use a trial-and-error approach to best understand how hybrid and wild species of rootstocks perform (Schwarz et al., 2010). Lee (1994) postulated that added vigor provided by interspecific hybrid rootstock is mostly associated with the production of plant hormones, and the size and ability of the root system to absorb and use water and nutrients. Others agree that increased vigor may be associated with an increased nutrient uptake or efficiency (Leonardi and Giuffrida, 2006; Savvas et al., 2009) in addition to water uptake (Djidonou et al., 2013; Martínez-Ballesta et al., 2010; Rivero et al., 2003b). Because of grafted plant's improved health and increased vigor, it may show a higher tolerance to foliar diseases and soilborne diseases, such as verticillium wilt (*Verticillium dahliae*), where genetic resistance has not been identified (King et al., 2008; Guan et al., 2012; Louws et al., 2010).

Studies also indicate that grafted plants may better tolerate abiotic stressors that might be a concern with intensified production such as temperature extremes (Rivero et al., 2003a and b; Schwarz et al., 2010), soil salinity (Colla et al., 2010; Fernández-García et al., 2004b; He et al., 2009; Rivero et al., 2003b; Savvas et al., 2011), drought and flooding (Schwarz, 2010), alkalinity (Savvas et al., 2010), nutrient deficiency, (Rivero et al., 2003b; Savvas et al., 2009; Schwarz et

al., 2013) and/or toxicity of nutrients, heavy metals, organic pollutants (Savvas et al., 2009, 2010; Schwarz et al., 2010). Weather is a major factor that causes crop stress in the Central U.S.; it can often be extreme and unpredictable with hot summers and early and late frosts. Rivero et al. (2003a) reported that when comparing nongrafted and grafted tomatoes grown at temperatures 25° C and 35° C, grafted plants had significantly higher biomass production and lower concentrations of phenolics than nongrafts in the higher temperature, indicating the role of certain rootstocks to reduce thermal stress. Although, reports have acknowledged particular cucurbit rootstocks as cold temperature tolerant, few have reliably identified tolerant tomato rootstocks (Schwarz et al., 2010; Rivero et al. 2003b).

Extensive research has and continues to focus of the tolerance of rootstocks to saline soil, which is critical to production in many parts of the world including the U.S. In moderate to high saline soil conditions, salt-tolerant tomato rootstock was found to increase photosynthetic and antioxidant activity as well as reduce cell damaging lipid peroxidase accumulation when compared to nongrafted plants (He et al., 2009).

Studies on nutrient and chemical uptake by grafted rootstocks have varied depending on rootstock and soil chemical or nutrient concentrations. Savvas et al. (2009) reported that grafting with the rootstock 'He-Man' had a differential effect on nutrient transportation in both high and low levels. For example, 'He-Man' was found to be tolerant of high levels of copper but increase the uptake of potassium (Savvas et al., 2009). The more we understand about rootstock behavior, the better we can select for qualities advantageous for the particular growing environment and for our food consumption. Urban areas may be exposed to more organic pollutants and heavy metals, and identifying rootstocks that inhibit or decrease their uptake could reduce the risk of exposure of toxins to humans when vegetables are grown in contaminated soils (Savvas et al.,

2010; Schwarz et al., 2010) However, the effect of grafting and potential profitability widely depends on the rootstock and scion selection and environmental conditions, and production practices (Lee et al., 1994, 1998, 2010; Louws et al., 2010; Savvas et al., 2011).

‘Maxifort’ and ‘Multifort’ are two tomato rootstocks that have been associated with additional vigor in a number of studies. Djidonou et al. (2013) reported that ‘Florida 47’ tomatoes grown in the open-field increased total and marketable yield when grafted onto interspecific hybrid rootstocks ‘Beaufort’ and ‘Multifort’. Furthermore, as the application of nitrogen increased from 112 kg/ha N to 280 kg/ha N, grafted plants continued to improve marketable fruit yield while nongrafted plants showed no significant increase in yield with additional N (Djidonou et al., 2013). The grafted tomato plants were also found to be more efficient than nongrafted plants at water and nitrogen uptake and performed best under 50% regime of recommended commercial irrigation application in Florida (Djidonou et al., 2013). In addition, ‘Maxifort’ rootstock consistently improved fruit yields when grafted with heirloom tomatoes and grown in a high tunnel at a commercial organic farm in North Carolina even though disease pressure from southern blight was much higher in one year than the other (Rivard et al., 2010a). In Kansas high tunnel trials, Masterson (2013) found that when compared to nongrafted plants, the tomato ‘BHN 589’ tomato scion grafted with ‘Maxifort’ or ‘Trooper Lite’ rootstock increased yield 18% to 126% when little to no disease pressure was evident. Furthermore, when discussing grafted plants, Lee (1994) noted that improved plant and fruit productivity is most evident when grafted plants are grown in the stressful environment often associated with protected agriculture.

In addition to fruit yield, grafting has the potential to affect fruit quality, depending on rootstock/scion selection and environmental growing conditions (Davis et al. 2008). The

literature is inconclusive as to whether grafting is advantageous or deleterious to tomato fruit physical, sensory, and nutritional quality (Davis et al., 2008; Roupael et al., 2010) and it may be more relevant to identify the impact made by specific rootstocks. Selected rootstock and scion pairs have shown to increase lycopene and β -carotene (Fernández-García et al., 2004b; Schwarz et al., 2013), firmness (Schwarz et al., 2013), and soluble solid content and titratable acidity in open-field growing conditions (Flores et al., 2010). Barrett et al. (2012b) observed no changes in sensory attributes when comparing grafted and nongrafted tomatoes grown in the open-field in Florida. Although, research by Zhao et al. (2007) indicates that nutritional quality of leafy greens is affected when grown in high tunnels compared to the open-field. However, limited research is available related to the quality of vegetables grown with grafted plants in the U.S. and in high tunnel production systems, particularly in the central region of the U.S.

As vegetable growers, especially urban and high tunnel growers, work to supply the demand of locally produced vegetables year-round with limited land and water resources, they often expose plants to a stressful growing environments such as extreme temperatures or undesirable soil moisture, salinity, pH, nutrients, and/or heavy metals (Savvas et al, 2010). Grafting vegetable may help high tunnel growers manage many abiotic as well as biotic stresses associated with the intensively cultivated soils and thus increase plant productivity in a limited space.

Challenges of Grafting

Although grafting with a vigorous and/or disease resistant rootstock can confer a number of benefits to a production system, there also are challenges related to grafting. Grafted plants add a significant cost both to produce and to purchase (Kubota et al., 2008; Lewis et al., 2014; Rivard et al., 2010b). Therefore, an unsuccessful grafting situation could occur when selected

rootstocks hinder plant productivity or fruit quality (Savvas et al., 2009). In some cases, the transplant itself could be unsuccessfully propagated or incompatibility can occur, which could potentially cause loss of profit for the grower. Lee (1994) identified a number of challenges related to grafting including: labor, cost of seeds, rootstock selection and compatibility, management of fertilizer, excessive growth, fruit quality, and scion rooting. Whether grafting your own plants or purchasing them from others, grafted plants will add to production costs. Rivard et al. (2010b) estimated that the production of a grafted tomato transplant costs \$0.49 to \$0.76 more than an equivalent-sized nongrafted plant. Although, there are several different added direct and indirect costs, such as supplies, labor, seedling production, and growing space required, Rivard et al. (2010b) found seed cost to be the highest contributor to the total increase in grafted transplant production, estimated at 33% to 52% additional cost. Although the initial cost of a grafted transplant is more than a nongrafted tomato plant, potential cost saving from pesticide use and overall increase in yields can justify the investment (Rivard et al., 2010b). Propagating your own grafted tomato plants has its own challenges, which are discussed in the section below.

As mentioned in the previous section, rootstocks are oftentimes selected for additional vigor to increase plant performance. However, vigorous rootstocks can increase plant uptake of nutrients and water resulting in more vegetative biomass; thus, potentially changing best management practices (Djidonou et al., 2013; Lee, 1994; Leonardi and Giuffrida, 2006; Rivero et al., 2003b). There is a limited understanding of irrigation, nutrient, and plant density recommendations for various rootstock and scion combinations.

Since grafting with selective rootstocks provides the ability to manage certain soilborne diseases, growers could potentially be attracted to reducing crop rotation intervals and become

too reliant on the technology (King et al., 2008; Louws et al., 2010). However, continuous cropping systems can potentially create a loss of tolerance and exacerbate other pest issues (King et al., 2008; Louws et al., 2010). Growers should continue crop rotation and diligent pest monitoring as well as consider rotation of rootstock varieties and/or species (King et al., 2008). Furthermore, researchers are working to identify and develop rootstocks that better manage soilborne pathogens and other stresses that are still problematic even with current technology.

Grafting in the Great Plains

One major barrier for U.S. growers looking to implement vegetable grafting is the low availability of grafted transplants (Kubota et al., 2008; Rivard et al., 2010b). Tens of millions of tomato plants are grafted in North America but a majority of them are propagated for large hydroponic greenhouse growers (Kubota et al., 2008). Because growers in the U.S. have limited options for purchasing grafted tomatoes, they may graft their own (Kubota et al., 2008). A survey of 265 fruit and vegetable growers at the 2014 Great Plain Growers Conference showed that 19% are using grafted vegetables but an additional 56% are interested learning more or incorporating grafted plants in their production (Rivard, unpublished data). Furthermore, 47% would prefer to graft their own while 25% would rather purchase plants (Rivard, unpublished data). Growers in the Central U.S. typically plant small batches of various cultivars for high tunnel and specialty production, which also contributes to the difficulty of purchasing grafted plants.

Traditionally, farmers in Japan and Korea grafted their own plants, but with an increase of demand and technology advances, cooperative groups organized to support grafting labor and post-grafting management (Lee, 1994). Many areas in the United States both rural and urban have formed farming or gardening organizations and/or associations to educate, grow food,

network, share resources, etc. Some of these organizations have communal propagation and growing greenhouse space, which could be an excellent opportunity to share grafting knowledge, technique, and management responsibilities, particularly in urban areas where greenhouse production space may be limited for growers. (Smit et al., 2001)

Most of the grafted plants around the world are now produced in large nurseries (Lee et al., 2010). With careful post-grafting management and a highly controlled growing environment, vegetable plants that are commercially grafted are often higher quality than those grafted on the farm (Kubota et al., 2008; Lee et al., 1998). As the interest in the technology increases, farmers buying from large propagators are concerned about a decrease in grafted transplant quality as a result of shipping over long distances, in addition to disease contamination/spread, and increases in cost (Kubota et al., 2008; Lee et al., 2010). Growers may benefit from buying locally propagated, grafted plants. If the demand continues to increase in the Central U.S., larger grafting operations that do custom grafting could be a profitable endeavor (Rivard et al., 2010b). However, with limited vegetable grafting knowledge and experience, propagators may be hesitant to invest in a technology that would require more time and money (Kubota et al., 2008). Therefore, simple, cost efficient techniques that result in high success rates are essential for more small-scale tomato growers or propagators to implement grafting technology in the U.S.

Propagation of Grafted Tomatoes

According to Lee et al. (2010), successful vegetable grafting can be described in 4 consecutive steps: (1) the choice of rootstock and scion species, (2) creation of a graft union by physical manipulation, (3) healing of the union, and (4) acclimation of the grafted plant. First a grower must prioritize the traits they are seeking with the use of grafted plants and choose a

rootstock accordingly (Lee et al., 2010). However, each rootstock may perform differently with paired scion so selection of both is critical to the overall success (Lee, 1994). Due to the numerous possible pairings, it is also likely that each may perform differently in various environmental conditions including soil type and biology (Guan et al., 2012). Flow of water and nutrients between rootstock and scion can be problematic when the vascular systems of the scion and rootstock fail to form a strong graft union (Martínez-Ballesta et al., 2010).

Splice Grafting Method

The “splice grafting method” also known as “Japanese top grafting” or “tube grafting method” is the most-commonly used technique for tomato because of its speed, low cost, and simplistic method for grafting young seedlings (Kubota et al., 2008; Oda, 1999; Rivard and Louws, 2006, 2011). It creates a strong vascular connection in the graft union and is recognized to generate a high quality and sturdy grafted seedling (Lee et al., 2010) that is capable of withstanding mechanical planting (Bausher, 2013). Although there are automated grafting robots, most grafting is done manually worldwide (Lee et al., 2010). Small-scale growers in the U.S. are grafting their own as a result of both necessity and interest (Kubota et al., 2008; Lewis et al., 2014; Rivard et al., 2010b).

In the splice grafting method, seedlings are grafted when the stem diameter reaches approximately 1.5 to 2.5 mm (Bumgarner and Kleinhenz, 2014; Rivard and Louws, 2006, 2011). It is important that the seedlings are healthy and uniform in size. The stems of the scion and rootstock are cut at a 70-degree angle and held together with a silicon clip (Bausher, 2013). The rootstock is cut below the cotyledons in order to prevent rootstock suckers, unexpected branches that could potentially form fruit (Bausher, 2011). However, it is not as clear whether the scion should be cut above or below cotyledons as advantages or disadvantages to grafting above or

below tomato scion cotyledons have yet to be reported. Most important, though, is that the scion and rootstock stem diameters are similar and cut angles are consistent for a high quality graft (Bumgarner and Kleinhenz, 2014). It takes approximately one to two weeks for the graft union to completely heal and one or more additional weeks of growth before planting (Bumgarner and Kleinhenz, 2014). While vascular tissue connects between the grafted scion and rootstock, propagators must manage the light, humidity, and temperature of the environment to avoid scion wilting, but promote cell division (Rivard and Louws, 2006, 2011; Bumgarner and Kleinhenz, 2014). The management of the post-grafting healing environment is one of the more challenging aspects of the grafting process especially for small-acreage growers with limited resources (Rivard, personal communication). During the grafting procedure, the excision of the scion's root system disrupts normal water uptake, and continuous transpiration will cause cell flaccidity and ultimately desiccation of the scion tissue. In addition, water stress can inhibit cell division (Taiz and Zeiger, 2002) and slow the healing process. Therefore, immediately after grafting, plants are placed in chambers with 80% or greater humidity and low light in order to decrease evaporation from the scion leaves (Bumgarner and Kleinhenz, 2014; Oda, 1999; Rivard and Louws, 2006, 2011). Using healing chambers that have a highly controlled environment post-grafting could reduce labor, in addition to increasing graft survival and transplant quality (Kubota et al., 2008; Lee et al., 2010; Oda, 1999). However, many small-acreage growers in the U.S. may have limited or no growing space with an automatically-controlled environment (Lewis et al., 2014), and typically utilize small greenhouses, converted high tunnels, or heated indoor space with lighting. For example, in a 2010 report, one small-scale grower in North Carolina built a small healing chamber inside of a heated high tunnel as compared to a modern greenhouse facility in Pennsylvania that had shading with cooling equipment and ebb and flood benches

(Rivard et al., 2010b). Developing methods for post-grafting management that improves grafted plant quality would further facilitate the ability of small-scale growers in the U.S. to graft their own plants.

Healing Chambers

Directly after the tomato seedlings are grafted, they must undergo a specific physiological process in order to heal and grow as a single plant. There are two distinct healing phases identified by Oda (2002). The first phase occurs in the first 4 days post-grafting and is associated with cell division of the tracheid (Oda, 2002). In the second phase occurring approximately between days 4 and 8, cell differentiation and elongation begins connecting the vascular scion and rootstock tissue (Fernández-García et al., 2004a; Oda, 2002). The entire process of graft union formation is fully developed in about 15 days (Fernández-García et al., 2004a). Meanwhile, the rest of the scion tissue must remain alive and turgid. In order to retain scion leaf and stem turgidity, grafts are typically moved into a healing chamber that has high humidity and low levels of light (Bumgarner and Kleinhenz, 2014; Oda, 1999; Rivard and Louws, 2006, 2011).

Healing chambers are highly variable especially when comparing large industry propagators to small-scale growers. Small-scale growers are recommended to use simplistic chamber designs such as a poly-covered frame inside a greenhouse (Bumgarner and Kleinhenz, 2014; Johnson and Miles, 2011; Johnson et al., 2011; Kubota et al., 2008; Masterson et al., in press; Oda, 1999; Rivard and Louws, 2006, 2011;). Larger commercial grafting nurseries, often available in other countries, use indoor facilities that are completely controlled including light, temperature, and humidity (Kubota et al., 2008; Lewis et al., 2014; Oda, 1999).

High humidity reduces the transpiration of water from the scion leaves and helps keep cells turgid. Although the plants can withstand some wilting, propagators want to avoid the permanent wilting point from which plants cannot recover turgidity (Taiz and Zeiger, 2002). It is recommended to cover frames with plastic, add standing water or capillary mats, mist plants, and/or use a cool-mister humidifier as means of keeping humidity high (Bumgarner and Kleinhenz, 2014; Johnson and Miles, 2011; Johnson et al., 2011; Masterson, 2013; Rivard and Louws, 2006, 2011). However, recently, research has shown potential for successful healing in an environment with lower humidity. For example, Johnson and Miles (2011) observed that a chamber with only shade cloth and no plastic covering resulted in 96% survival rate of grafted tomato plants after 7 days with only 53% average relative humidity (RH). Work by Masterson et al. (in press) also showed that chambers with lower RH (average 69%) produced similar survival rates as those with very high (>90%) RH.

By reducing water stress in the scion, it may be feasible to utilize healing chambers that maintain lower RH with good success even for new growers with limited experience and facilities. Masterson et al. (in press) reported that completely defoliating tomato seedlings as a strategy to decrease water stress during healing increased grafted plant survival rates when compared to the standard method, with no leaf removal. However, removal of the apical meristem (or shoot) did not affect survival rates when compared to standard method (Masterson et al., in press). Furthermore, shoot removal is often practiced when propagating grafted tomatoes, particularly for greenhouse growers that use the European string trellis training systems for fruit production (Kubota, 2008). Although nongrafted plants are pruned to a single leader, plants grafted with a vigorous rootstock are typically trained to two leaders as a way to reduce costs and take advantage of vigorous rootstocks (Besri, 2003). In the report by Masterson

(2013), not only did shoot removal show no advantages in propagation, its necessary re-growth period hindered early field performance in some rootstock and scion combinations.

The optimum temperature during the graft union healing process is 21° to 30° C (Bumgarner and Kleinhenz, 2014; Oda, 1999; Rivard and Louws, 2006, 2011). Light and humidity also can have an effect on temperature within the healing chamber. Heat stress on plants exacerbates transpiration thereby increasing plant wilting, and temperatures exceeding 45 °C can cause tissue death (Taiz and Zeiger, 2002). Normally, plants cool their leaves through evaporative cooling by transpiration (Taiz and Zeiger, 2002). Therefore, setting a high humidity slows transpiration rates, and causes leaf temperatures to rise (Taiz and Zeiger, 2002). Smaller leaves are able to remain closer to air temperature even with reduced transpiration as they have a higher surface area to facilitate heat exchange (Taiz and Zeiger, 2002). In addition to reducing the effect of evaporative cooling within the leaf, enclosing the chambers with plastic can cause an increase in temperature, particularly if they are located inside of a greenhouse with direct light. Growers are recommended to ventilate healing chambers several times daily (Rivard and Louws, 2006, 2011) and some cut ventilation holes on top of the chamber to release excess heat (Rivard, personal communication). Both Johnson and Miles (2011) and Masterson et al. (in press) reported that chambers with only shade cloth exhibited cooler temperatures than the chambers that had shade cloth and plastic film.

Plant propagators significantly reduce light to grafted tomatoes during the first 4 days of healing to reduce transpiration and water stress (Bumgarner and Kleinhenz, 2014; Rivard and Louws, 2006, 2011). Low light closes stomata which decreases loss of water vapor but also gas exchange used in photosynthesis (Taiz and Zeiger, 2002). As stated previously, shading can help reduce chamber temperatures. However, light helps to encourage the healing process (cell

division) so it is vital to reintroduce some light soon after grafting to support cell division and resume normal photosynthesis and growth. Healing chamber light can be controlled by using shade cloth, tarps, styrofoam, etc. Many larger grafting nurseries utilize indoor healing chambers, which have lighting systems that can be enabled throughout the healing process (Kubota et al., 2008; Lewis et al., 2014). Nobuoka et al. (2005) reported that using blowers to circulate air within high light intensity and high humidity healing chamber hastened the healing of the graft union. Air circulation may help with cooling leaf temperatures in high humidity chambers.

Balancing light, humidity, and temperature for the ideal post-grafting environment is oftentimes difficult for growers, particularly those that have little experience with propagation methods or facilities to enable the proper environment. Healing chamber design in addition to the facilities where the healing chamber is physically located represent different ways of approaching the problem. There are a number of ways that grafted transplants can lose quality other than simply not surviving the grafting procedure. Propagators with little experience grafting oftentimes grow plants that have elongated within the healing chamber and are not sturdy enough to survive planting in the open-field. Or, plants may have been successfully grafted, but are stunted due to poor or slow graft union formation.

Adventitious Rooting of Scion

One problem that could be related to the healing chamber environment is the formation of adventitious roots (AR) on the stem of the scion. Growers are recommended to avoid adventitious rooting on the scion as they can form a “bridge” for soilborne pathogens to infect potentially-susceptible scion tissue (King et al., 2008; Rivard and Louws, 2006, 2011). Lee (1994) reported that some AR formation from the scion is common, but research results are

mixed on how they may affect the plant's vigor and stress tolerance. However, it is more commonly assumed and even observed that AR from the scion can increase the potential for soilborne disease even with resistant rootstocks (King et al., 2008; Lee et al., 2010; Rivard and Louws, 2006, 2011). Furthermore, AR can dislodge the scion tissue from the grafting clip or root into directly into the growing media, thereby resulting in loss-of-function by the rootstock.

Some growers and extension educators have associated production of AR with the high humidity levels experienced in the healing chamber (Johnson et al., 2011). However, literature suggests that the plant wound response process during grafting may promote the development of AR as well. Adventitious roots initiate from stem or leaf cells capable of differentiation into root initials (Geiss et al., 2010). The wounding that occurs during vegetative propagation of cuttings is similar to the excising the scion for grafting. Therefore, research in vegetative propagation for vegetables may help us understand how wounding promotes AR, as often desired in vegetative production, and how we may prevent them in grafting. Their formation is a normal occurrence in many plants, but it can also be promoted by many endogenous and exogenous factors. (da Costa et al., 2013; Geiss et al., 2010). Exogenous factors are most often related to the surrounding environment such as wounding, nutrition availability, light, and temperature, but also fungal and bacterial biota that promote rooting (Geiss et al., 2010). Further research focuses on understanding the role of endogenous phytochemicals and phytohormones in adventitious root formation as well as how it relates to the genetic disposition of the plants to promote adventitious roots (Geiss et al., 2010).

Several authors have described the formation of AR in many different phases. Kevers et al. (1997) classified the development of adventitious roots into 3 distinct steps: induction, initiation, and expression. The induction phase is the phytochemical events that lead up to the

initiation phase of cell division, and ultimately root growth in the expression phase. De Klerk (1999) also identified a preliminary step before the induction phase where cells dedifferentiate in response to wounding. However, for basic communication, it can be described in two phases: the induction phase (including any dedifferentiation phase) and formation phase (initiation and expression phases) (da Costa et al., 2013).

The induction phase of AR can begin within 24 hr. of wounding (Kevers et al, 1997). Wounding causes several reactions in plants. It can lead to closure of stomata, which decreases transpiration and gas exchange (Taiz and Zeiger, 2002). It also causes many endogenous chemical responses including the accumulation of the hormone auxin at the wounding site (da Costa et al., 2013). Auxin is produced in meristematic tissue in the shoots and young leaves and flows basipetally (Taiz and Zeiger, 2002). Within the first hours after wounding, auxin will be transported towards the roots from the leaves, particularly the young leaves, and meristematic tissue directly above the wounding site (Katsumi et al., 1969; Maldiney et al., 1986; Nordström and Eliassum, 1991). Auxins have been associated with the development of AR in shoot cuttings of pea (*Pisum sativum*) (Nordström and Eliassum, 1991), cucumber (*Cucumis sativus*) (Katsumi et al., 1969) and tomato (*Solanum lycopersicum*) (Maldiney et al., 1986). Although increased levels of auxin at the wound site are vital in the regeneration of vascular tissue, higher levels seem to promote adventitious rooting (da Costa et al., 2013; Katsumi et al., 1969; Maldiney et al., 1986; Nordström and Eliassum, 1991). Oftentimes vegetative propagators will apply exogenous auxin to the end of a cutting to promote AR formation (da Costa et al., 2013). Cytokinin is another plant hormone associated with vascular regeneration; it is mostly synthesized in the roots and transported apically (Maldiney et al., 1986; Taiz and Zeiger, 2002). The ratio of auxin to cytokinin has been found influence the plant tissue regeneration (Eklöf et

al., 1997). During the induction phase, a high ratio of auxin to cytokinin stimulates adventitious root formation and xylem regeneration (Kevers, 1997; Taiz and Zeiger, 2002). In contrast, a low auxin to cytokinin ratio initiates shoot formation and phloem regeneration (Taiz and Zeiger, 2002). Furthermore, the concentrations of auxin and cytokinin can affect each other's metabolism and transportation (Eklöf et al., 1997).

Maldiney et al. (1986) reported that root initials of tomato cuttings were visible at day 4 after wounding indicating the beginning of the formation phase. The prior 3 days signifies the induction phase where cells are dedifferentiating into root initial tissue. Furthermore, Maldiney et al. (1986) found that within the first 3 days, auxin levels gradually increased at the wounding site while cytokinin levels dramatically decreased in the first 24 hours and consistently stayed low. By day 5, auxin levels had decreased back to initial levels and cytokinins were noticeably increased (Maldiney et al., 1986). The 3-day induction phase of AR on tomato cuttings coincides with the first healing phase of the graft union prior to vascular connection. Therefore, the first 4 days post-grafting are probably the most influential for cellular reconstruction whether vascular regeneration or root initials. Even with decades of research in genetics, phytochemical and phytohormone influence in plant development, researchers still lack a full understanding of AR and inhibition (Geiss et al., 2010). Auxin and cytokinin are the most notably known hormones to promote AR, but other phytochemicals and major hormones can influence either the initiation or inhibition of AR, especially in response to wounding or water stress (da Costa et al., 2013; Geiss et al., 2010). Although it's not clear how exactly hormones play a role, there is ample evidence to suggest that the wounding process that occurs during grafting is contributing to the formation of AR and that some of these hormones are produced in the leaves (Maldiney et al., 1986).

Environmental factors such as available nutrition, humidity, temperature, and light could also be involved in the induction and formation of AR (Geiss et al., 2010) on grafted tomato transplants. Many researchers and growers have noted anecdotally the prevalence of AR when healing chambers are kept overly-humid (Johnson et al., 2011), when thermal stress events (overheating), or poor grafting technique is utilized. However, little research addresses the prevention of AR on grafted tomato transplants. Lee (1994) mentions that AR can be avoided with careful cultural management but without further explanation of the specific practices required. Although one extension publication mentions grafting above the cotyledons to prevent AR (Rivard and Louws, 2006), other publications say to excise scion either above or below cotyledons (Rivard and Louws, 2011; Bumgarner and Kleinhenz, 2014).

Defoliating scions prior to grafting is a simple technique that can increase graft survival (Masterson, in press). It decreases leaf transpiration and water stress during the healing process, which may effect AR formation (Bumgarner and Kleinhenz, 2014). In addition, the leaves, cotyledons, and apical meristem above the wounding site are a source of auxin that is transported toward the graft union (Katsumi et al., 1969). Katsumi et al. (1969) showed that the removal of different proportions of cotyledons, but intact apical meristem on cucumber cutting reduced auxin levels at the basal site and decreased AR formation. Therefore, leaf removal of the scion immediately prior to grafting could possibly affect the formation of AR while the graft union heals. However, a reduction in leaf area could also decrease photosynthesis and consequently hinder transplant growth and quality (Bumgarner and Kleinhenz, 2014).

Research Objectives

High tunnel production provides unique opportunities for urban and peri-urban growers to increase productivity and quality of tomato crops, particularly for those with limited growing spaces. However, the intensive cultivation and microclimate can also induce issues with soil, nutrient, and disease management. Grafting with selected rootstocks could be a mechanism to reduce damage from biotic and abiotic stressors within both field and high tunnel systems. However, with inadequate availability of grafted plants in the Central U.S., growers may be unable to adopt this technology and those with limited greenhouse facilities could face difficulties propagating grafted tomato plants. Therefore, it is clear that further information on rootstocks that increase productivity and profitability in high tunnel systems as well as simplified propagation techniques for small-scale growers and propagators is greatly needed. Therefore, our research objectives were the following:

- To evaluate if grafted tomatoes are advantageous by increasing yield and/or biomass for urban and peri-urban high tunnel production in the Central U.S.;
- To identify tomato rootstocks that increase productivity in yield and/or biomass when little to no soilborne disease pressure in high tunnels is evident; and
- To assess how leaf removal of the scion during grafting affects adventitious rooting on the scion, grafted transplant quality, and early transplant growth and development.

Chapter 2 - Grafting to Increase Tomato Productivity for High Tunnel Production in the Central U.S.

Introduction

High tunnels are unheated, polyethylene film-covered greenhouse structures used around the world to protect crops from unfavorable environmental conditions (Lamont, 2009; Wells and Loy, 1993). In addition to crop protection, they allow fruit and vegetable growers to extend growing seasons at a low cost (Lamont, 2009; Wells and Loy, 1993). In the United States, research on high tunnel production has been particularly motivated by consumer-demand for more local produce (Carey et al., 2009). Current efforts encourage high tunnel use in urban growing environments, such as for urban farms, schools, non-profit organizations and community gardens (Lamont, 2013).

In the Central United States growing region, interest surrounding high tunnel production continues to grow (Carey et al., 2009; Knewton et al., 2010a). At the 2014 Great Plains Growers Conference (GPGC) in St. Joseph, MO, surveyed vegetable, fruit, and flower growers (n=265) reported that currently, 42% use high tunnels, 23% using high tunnels and want to add more, and 18% want to add them into their production (Rivard, unpublished data). Tomato (*Solanum lycopersicum*) is the most popular high tunnel crop (Carey et al., 2009; Knewton et al., 2010a; Lamont, 2009). However, profitability relies heavily on production management, marketability, and environmental factors (Blomgren and Frisch, 2007; Conner et al., 2010; Everhart et al., 2009; Galinato and Miles, 2013; Sydorovych et al., 2013; Waterer, 2003). In addition to extending the growing season, high tunnels can protect crops and particularly fruiting vegetables from other environmental factors such as hail, rain, sun, and wind (Wells and Loy, 1993).

Keeping tomato foliage dry and regulating water application through drip irrigation in high tunnels helps reduce the risk of foliar and fruit diseases such as early blight, tomato spotted wilt virus, and gray leaf spot as well as abiotic disorders associated with soil moisture fluctuations such as fruit cracking and blossom end rot (Rogers and Wszelaki, 2012; O'Connell et al., 2012). Nevertheless, the high tunnel microclimate and intensive cultivation creates its own biotic and abiotic stresses, and challenges growers to adjust management for growing in restricted space (Blomgren and Frisch, 2007; Everhart et al., 2009; Galinato and Miles, 2013; Knewton et al., 2010a).

Worldwide, high tunnel growers graft tomato with rootstocks resistant and/or tolerant of diseases and abiotic stresses that are commonly associated with intensive vegetable production in protected agriculture (Lee et al., 2010). Vegetable grafting began in Japan and Korea with cucurbit rootstocks for melons in the late 1920's to increase yields and managing diseases such as fusarium wilt (*Fusarium oxysporum*) (Lee et al., 1994; Kubota et al. 2008). Later in the 1950's and 1960's, grafters further applied the technology to cucumbers and solanaceous crops eggplant and tomato (Oda, 1999, 2002). In North America, producers started adopting grafting for large hydroponic tomato greenhouse operations around the 1990's (Kubota et al., 2008). In the United States, vegetable grafting was slower to be adopted as crop rotation could be practiced with wide availability of land (King et al., 2008; Lee, 1994). However, as the soil fumigant methyl bromide began phasing out in the United States and the attention in sustainable and organic agriculture increased, grafting research and utilization became even more relevant (Kubota et al., 2008; Louws et al., 2010; King et al., 2008). Furthermore, as land value continually increases, especially in urban and peri-urban areas, and with the rapid expansion of high tunnels, more small-scale growers are interested in grafting for vegetable production in the U.S. (Lewis et al.,

2014). We also surveyed growers at the 2014 GPGC on their interest and usage of grafted plants. Results show that 19% of survey participants (n=265) (65% of which are growing in high tunnels) are using grafted plants, but an additional 56% are interested learning more or incorporating grafted plants into their production (Rivard, unpublished data).

In the southeast areas of the United States, grafting is used to manage many devastating soilborne diseases of tomato: fusarium wilt (caused by *Fusarium oxysporum* f.sp. *lycopersici*) (Rivard et al., 2008); bacterial wilt (caused by *Ralstonia solanacearum*) (McAvoy et al., 2012; Rivard et al., 2012); southern blight (caused by *Sclerotium rolfsii*) (Rivard et al., 2010a); and root-knot nematodes (*Meloidogyne* spp.) (Rivard et al., 2010a; Barrett et al., 2012c). For example, Rivard et al. (2012) reported that in moderate disease pressure from bacterial wilt grafting ‘Celebrity’ scion onto ‘RST-04-105’ increased total fruit yield in weight by nearly 80%. Soilborne pathogens often require long-term crop rotations for avoidance, but integrating grafting into pest management may help high tunnel growers with limited space for crop rotations (King et al., 2008; Louws et al., 2010; Rivard and Louws, 2008). However, with the Central U.S., many of the soilborne diseases that plague tomato crops in the southeast U.S. are either not present such as bacterial wilt or southern blight or less problematic such as fusarium wilt. The regional differences are mostly likely due to colder winters and larger areas for proper crop rotation in open-fields.

In addition to disease resistance, grafting tomato with select rootstocks can lead to tolerance of abiotic environmental stresses that may be associated with high tunnels such as high and low temperature, water stress, (Rivero et al., 2003a and b; Schwarz et al., 2010), soil salinity (Colla et al., 2010; Fernández-García et al., 2004b; He et al., 2009; Rivero et al., 2003b; Savvas et al., 2011), soil alkalinity, and nutrient deficiency or toxicity (Savvas et al., 2010; Schwarz et

al., 2010, 2013). Moreover, researchers are working to identify Solanaceae and Cucurbitaceae rootstocks that tolerate uptake of heavy metals and organic pollutants contaminating potential agricultural soils (Savvas et al., 2010; Schwarz et al., 2010). However, stress tolerance and crop performance depends on the rootstock and scion selection as well as the environmental conditions (Guan et al., 2012; Lee, 1994; Savvas et al., 2010). Furthermore, without a clear understanding of rootstock genetics, most of the breeding and research is trial-and-error based (Savvas et al., 2010). Therefore, much of abiotic stress tolerance is associated with the vigorous attributes of particular rootstocks (Guan et al., 2012; Savvas et al., 2010). Vigorous rootstocks increase the uptake and use efficiency of nutrients and water (Leonardi and Giuffrida, 2006; Djidonou et al., 2013), in addition to phytochemical production and distribution (Lee, 1994). As a result, plants grafted onto vigorous rootstocks can have increased total plant biomass, fruit yield, and fruit size compared to nongrafted plants (Barrett et al., 2012c; Masterson, 2013). Grafted plants with increased vigor may help plants tolerate foliar and soilborne diseases like tomato spot wilt virus, tomato yellow leaf curl virus, and verticillium wilt (*Verticillium dahliae*) (Guan et al., 2012; Louws et al., 2010; Rivard et al., 2008; Rivero et al., 2003b).

Because the added costs of a grafted transplant ranges from \$0.46 to \$1.12 per plant, a certain level of yield increase is required to offset inputs costs and maximize profit, particularly in regions where soilborne diseases may not be a top concern for growers (Rivard et al., 2010b). Increased fruit yields would help offset the increased cost of propagated grafted tomato plants. However, few studies have reported, which rootstocks may increase vigor for tomato plants grown in high tunnels in the Central U.S. Therefore, the objective of this work was to identify tomato rootstocks that increase productivity in high tunnels with little to no pressure from soilborne diseases.

Materials and Methods

High tunnel trials were conducted in 2013 and 2014 at three northeast Kansas commercial farms in addition to the Kansas State University Olathe Horticulture Research and Extension Center (OHREC). All sites had no known history of soilborne diseases. Transplants were propagated and grafted at OHREC in a quonset-style greenhouse. Each trial was arranged in a randomized complete block design (RCBD) with at least four replications. All nongrafted and self-grafted plants were the tomato cv. BHN 589 (BHN Seed; Immokalee, FL), which is a hybrid red slicer tomato variety with sizeable determinate plant growth that performs well in high tunnels. It is known for its high yield of quality fruit with long shelf life. Rootstocks being evaluated and grafted with 'BHN 589' scion included the following: 'RST-04-106' (DP Seeds; Yuma, AZ); 'RT 1028' (BHN Seed; Immokalee, FL); 'Maxifort' (De Ruiter; St. Louis, MO), 'Multifort' (De Ruiter; St. Louis, MO), 'DRO 131' (De Ruiter; St. Louis, MO), 'Arnold' (Syngenta; Greensboro, NC), and 'Colosus' (Rijk Zwaan; De Lier, The Netherlands). We chose these particular varieties because they were common or newly developed rootstocks recommended by breeders. Both rootstock varieties 'RST-04-106' and 'RT 1028' are suggested for possible bacterial wilt management, a persistent soilborne disease problematic and destructive for tomato growers in the southeast U.S. 'Arnold' is another popular rootstock variety because it is effective at managing diseases caused by soilborne pathogens like *Colletotrichum coccodes* (Gilardi et al., 2014) and *Phytophthora spp.*, whereas other commercially-popular rootstocks like 'Maxifort' are more susceptible (Gilardi et al., 2013). 'Maxifort' and 'Multifort' are two similar interspecific hybrid rootstocks known for both disease management and increased biomass and fruit yield. 'Maxifort' is reported to provide resistance to soilborne diseases like fusarium wilt (Rivard et al., 2008) and southern blight, and has shown

evidence of tolerance to pathogens like root-knot nematodes (Rivard et al., 2010a) and *Verticillium dahliae* (race 2) (Louws et al., 2010). We were recommended 'DRO 131' rootstock for our trials but with limited seed availability to growers, we replaced it with 'Colosus' rootstock in 2014.

Based on the available growing space and data collected in previous years, we selected the different rootstock treatments for each farm individually, and they are identified in the description of each site. The Johnson County Farm was originally was a separate project in 2013 and so the treatment selection was dissimilar to the other three trial sites, but was adjusted in 2014 to be better related. In addition, because 'RT 1028' rootstock performed poorly in 2013, it was replaced in 2014 by 'Arnold' at both the Douglas County Farm and Wyandotte County Farm. All trials included a nongrafted 'BHN 589' control as well as a self-grafted 'BHN 589' treatment except for the 2013 Johnson County Farm. The self-grafted 'BHN 589' scions were grafted onto their original root systems in order to observe at what degree, if even, the wounding action and healing of the graft potentially influences plant growth and yield. At approximately 1.5 to 2 mm stem diameter, plants were grafted using recommended splice grafting (tube grafting) method and chamber management procedures, including sanitation care with soilless media, propagation and transplant plastic trays, chambers and grafting tools and gloves to prevent disease spread (Rivard and Louws, 2011). Nongrafted plants were kept in cool room in the greenhouse with a temperature range set at 12 °C to 30 °C both day and night to maintain plant growth while grafted planted healed in chamber for 7 days. Post-grafting, we moved both grafted and non-grafted transplants back to normal greenhouse conditions for complete graft healing and to slowly acclimate them to lower temperatures until planting dates. At time of planting for each high tunnel site in 2014, approximately 10 or more random 6" deep soil

samples were taken within the growing areas, mixed homogeneously, and submitted to the K-State Soil Testing Lab (Manhattan, KS) for analysis of pH, organic matter, EC, and NO₃-N.

High Tunnel Trial Sites

Olathe Horticulture Research and Extension Center

The research farm, OHREC, is located in Johnson County, KS (38.884347 N, 94.993426 W) and has Chase silt loam soil (pH= 6.3) and uses organic practices. Six replicates were planted in six identical 6.1 x 9.8 m Quonset-style high tunnels with one half of each high tunnel used for one replication of the trial. For two years prior to the 2013 trial, the high tunnels had no polyethylene cover and winter rye (*Secale cereal*) was utilized as a cover crop. In late April 2013, four weeks prior to planting, the cover crop was chopped down and incorporated it in the top 4 to 6 inches of the soil using a tiller. Prior to planting, the high tunnels were covered with 6-mil poly-film, but late timing of the polyethylene application caused the 2013 trial planting date to be delayed nearly a month compared to planting date in 2014. The four treatments in 2013 were nongrafted and self-grafted plants as well as plants grafted with 'RST-04-106' and 'RT 1028' rootstocks. Plots sized 4.1 m x 1.5 m were oriented east to west, similar to the high tunnels. Within each plot, nine plants spaced 45.7 cm apart were planted on 31 May 2013. The opposite side of the tunnel was planted in buckwheat (*Fagopyrum esculentum*) cover crop for the summer and oats (*Avena sativa*) during the fall/winter. In 2014, the cover crop was incorporated similarly and tomato transplants planted on 21 April. The orientation of the rows in 2014 was north to south, which allowed us to include 5 treatments: nongrafted and self-grafted plants and plants grafted with 'RST-04-106', 'RT 1028', and 'Maxifort' rootstocks. In each 2.7 x 1.5 m plot, six plants were set 45.7 cm apart within the row. At the time of planting in the 2014 trial,

soil sample results showed the following: pH = 7.2; organic matter = 4.2%; EC = 0.538 mS/cm; and NO₃-N = 17.4 ppm. In both years, organic pelleted chicken fertilizer, Chickity Doo Doo (Unlimited Renewables, LLC; Onalaska, WI), 5N-1.5P-2.3K, was applied at a rate of 112 kg N per hectare at planting. Management practices included drip irrigation, fabric mulch, a stake-and-weave trellis, natural predator releases and organic pesticides. Fruit was harvested once to two times per week from 17 July to 23 Oct. 2013 and 17 June to 10 Oct. 2014.

Wyandotte County Farm

The Wyandotte County, KS site (39.057955, -94.678209) was at a commercial certified organic urban farm in Kansas City, KS with Lagoda silt loam and Marshall silt loam soil. Gibb's Road Farm is a both a working and teaching demonstration market farm for the non-profit organization Cultivate Kansas City (www.cultivatekc.org). The trial was planted in a 7.3 x 29.3 m quonset-style high tunnel with four replicated blocks that were planted within the outer two (of four) rows of the high tunnel. Experimental plots were 2.3 x 1.5 m in length and consisted of five plants spaced 45.7 cm apart. Approximately six buffer plants were planted on both ends of the tunnel and an empty space was left between plots. Treatments in the 2013 trial included nongrafted and self-grafted plants and plants grafted with 'RST-04-106' and 'RT 1028' rootstocks, and in the 2014 trial, treatments included nongrafted and self-grafted plants and plants grafted with 'RST-04-106' and 'Arnold' rootstocks. In both 2013 and 2014, organic pelleted chicken fertilizer, Chickity Doo Doo (Unlimited Renewables, LLC; Onalaska, WI), 5N-1.5P-2.3K, was applied at planting at a rate of 143 kg N per hectare. At time of planting in 2014, soil tests indicated the following: pH = 7.0; organic matter = 6.7%; EC = 3.52 mS/cm; and NO₃-N = 133.3 ppm. The trials were planted on 12 and 7 April in 2013 and 2014, respectively. Cultural management practices included drip irrigation, straw mulch, and a stake-and-weave trellis. Fruit was harvested

once to two times per week from 25 June to 7 Oct. 2013 and 20 June to 24 Sept. 2014. In 2014, trial was terminated without final data of harvested fruit number and weight.

Douglas County Farm

Common Harvest is a certified organic urban farm located in Douglas County, KS (38.962799, -95.209216) in the city of Lawrence, KS. In 2013, the four replicates were located on the outer two rows of two different 7.3 x 29.3 m quonset-style high tunnels (2 reps each). On 23 Apr. 2013, five plants of each treatment were planted at 45.7 cm in-row spacing and the plots were 2.3 x 1.5 m. The rootstocks tested at this location were 'RST-04-106' and 'RT 1028' in 2013 and 'Arnold', 'RST-04-106', and 'Maxifort' in 2014. In 2013, approximately 1 cm of municipally produced composted was evenly broadcasted as a thin layer and tilled into the soil inside the tunnels as a pre-plant fertilizer. In addition, about a half cup of a mix of kelp meal, feather meal, calphos, and greensand was added to holes at time of transplant. In 2014, all four replications were located in one high tunnel and two replications were planted to each of the middle two rows. The 2014 trial was planted on 11 April, and the same plot size and plant numbers were used. Two buffer plants were planted on ends of each row of the tunnel and an empty space left was between plots in both years. Soil sampled at time of planting in 2014 reported the following: soil pH = 6.8; organic matter = 4.5%; EC = 5.67 mS/cm; and NO₃-N = 43.1 ppm. In the 2014 trial, the plants were side-dressed with organic pelleted chicken fertilizer, Chickity Doo Doo (Unlimited Renewables, LLC; Onalaska, WI) 5N-1.5P-2.3K, at a rate of 103 kg N ha⁻¹ ten weeks after planting. Other management practices included drip irrigation, a stake-and-weave trellis and straw mulch in 2013 and fabric mulch in 2014. Fruit was harvested once to two times per week from 20 June to 9 Oct. 2013 and 23 June to 29 Sept. 2014.

Johnson County Farm

Gieringer's Orchard is a conventional peri-urban fruit and vegetable farm located in Johnson County, KS (38.76473, -95.008022) and it has Sibleyville loam soil (pH=6.8). The farm used a 35 x 9 m Gothic arch style high tunnel with its single layer of plastic also covered with 30% shade cloth applied 3 weeks prior to first harvest. The tunnel layout consisted of four double rows (8 rows total) with 0.5 m row spacing within the double rows and 1.4 m spacing between the double rows. Each replication was planted in one of the four centrally located rows with at least five or more buffer plants at the ends of the rows. On 5 Apr 2013, six plants spaced 61 cm apart were planted in 3.7 x 0.5 m plots with an empty space was left between plots for each of the six treatments: nongrafted plants and plants grafted onto 'Maxifort', 'Multifort', 'DRO 131', 'Arnold', and 'RT 1028' rootstocks. An. On 2 Apr. 2014, six plants were planted for the seven treatments: nongrafted and self-grafted plants and plants grafted onto 'Maxifort', 'Arnold', 'RT 1028', 'RST-04-106', and 'Colosus' rootstocks. Instead of leaving an empty space, one yellow tomato fruit plant var. BHN 781 was planted between plots for visual separation of treatments during harvest. The grower's soil management features incorporating a 5 cm layer of composted cow manure with bedding into the soil every other fall season and fertigating crops once approximately 3 weeks after planting with a complete fertilizer. Compost was added in the fall of 2013. In 2014, pre-plant soil sample indicated the following: pH = 6.6; organic matter = 9.2%; EC = 3.32 mS/cm; and NO₃-N = 230.5 ppm. Management practices included drip irrigation, fabric mulch, a stake-and-weave trellis, and conventional pesticide applications. Fruit was harvested once per week from 3 July to 21 Oct. 2013 and 17 June to 7 Oct. 2014.

Data Collection and Analysis

At each harvest, tomato fruit was sorted into marketable and unmarketable grades, counted, and weighed. Marketability was based upon on-farm standards for direct retail sales and unmarketable fruit commonly exhibited significant cracks, insect pest and fruit rot damage, and/or blossom end rot. During the last harvest, all fruit larger than 5 cm were counted and weighed, except for the Wyandotte County Farm in 2014. One whole plant was destructively sampled from each experimental plot at the end of the season in order to assess shoot biomass. Plant samples were dried at 70 °C for at least 72 hrs. and then weighed. Anecdotal observations took place throughout the season for any symptoms of soilborne disease; no such evidence was discovered throughout all eight trials. The average fruit size for both total and marketable yields were determined by dividing the yield in weight divided by the tomato count. In addition, by dividing the marketable yield by the total yield, we assessed the % marketability for both fruit weight and number. All data were analyzed on a per plant basis using PROC GLIMMIX (SAS 9.2; SAS Institute, Cary, NC USA). Fruit weight, fruit size, % marketability by weight, and dry plant biomass were compared each year independently for each site using Fisher's protected LSD with a $P < 0.05$. Statistics comparing LSD for fruit count and % marketability by fruit number used the Poisson model with $\alpha = 0.05$.

Results

Olathe Horticulture Research and Extension Center

Results for OHREC can be seen in Table 2-1. There were no differences in any yield or plant growth parameters in 2013, and in 2014, there were significant differences in all yield measures except % marketability. Plants grafted with 'RT 1028' and 'RST-04-106' rootstocks

had similar marketable and total fruit number and weight to the nongrafted and the self-grafted plants in both 2013 and 2014. Similarly, total plant biomass was not significantly affected by the utilization of these two rootstocks. However, in 2014, plants grafted with ‘RST-04-106’ rootstock had larger total fruit size for total yield when compared to the nongrafted plants, but not the self-grafted ones. Furthermore, in 2014, plants grafted with ‘Maxifort’ rootstock produced significantly more total and marketable fruit in number and weight as well as larger fruit size and total plant biomass than the nongrafted and self-grafted plants. Compared to the nongrafted plants, plants grafted with ‘Maxifort’ rootstock increased the total and marketable fruit weight by 40% and increased the average size of fruit by 19 g.

Wyandotte County Farm

Results for Wyandotte County Farm are presented in Table 2-2. In 2013, grafted plants did not increase any yield or percent marketability in both fruit number and weight when compared to nongrafted and self-grafted plants. In 2014, though, total and marketable yield number and weight did increase for selected grafted plants when compared to either nongrafted or self-grafted plants. Fruit size and total plant biomass was similar for all treatments in both years. Although, in 2013, plants grafted with ‘RT 1028’ or ‘RST-04-106’ rootstocks did not increase any yield or growth parameters, in 2014, plants grafted with ‘RST-04-106’ rootstock did significantly increase total and marketable fruit numbers compared to nongrafted and self-grafted plants. ‘Arnold’ rootstock also increased the total and marketable fruit number compared to both nongrafted and self-grafted plants. Furthermore, plants grafted with ‘Arnold’ rootstock increased total fruit weight by 65% and marketable fruit weight by 60%, which was significantly more than the self-grafted plants but similar to the nongrafted plants. Only ‘RT 1028’ rootstock decreased marketable fruit number and percent marketability by weight in 2013.

Douglas Co. Farm

Results for Douglas Co. Farm are presented in Table 2-3. Grafted plants did not significantly increase any yield or plant growth parameters in 2013. In 2014, differences between grafted and nongrafted plants are observed in total fruit number and weight, marketable fruit weight, and plant biomass. In 2013, ‘RST-04-106’ rootstock produced lower total and marketable fruit compared to nongrafted and self-grafted plants. In addition, the weight of marketable fruit decreased for plants grafted on both ‘RST-04-106’ and ‘RT 1028’ rootstocks when compared to nongrafted plants, which also significantly decreased the overall marketability of fruit (by weight) for plants grafted with ‘RST-04-106’ rootstock. Moreover, in contrast to the 2013 trial, the marketable yield and % marketability were similar for plants grafted with ‘RST-04-106’ rootstock compared to nongrafted and self-grafted plants and also increased total fruit numbers compared to nongrafted plants. ‘Arnold’, and ‘Maxifort’ rootstocks also had significantly higher total fruit numbers as well as total and marketable fruit weight compared to the nongrafted plants. The total fruit weight for plants grafted ‘Arnold’ and ‘Maxifort’ rootstocks increased by 45% and 59%, respectively, compared to nongrafted plants, but showed similar results to self-grafted plants. Plants grafted to ‘Maxifort’ rootstock also had higher total plant biomass than the nongrafted and self-grafted plants. However, grafting with ‘Maxifort’ rootstock reduced marketability (by number) compared to nongrafted and self-grafted plants. Interestingly, the self-grafted plants had higher total fruit number compared to nongrafts in the 2014 trial.

Johnson Co. Farm

Results for Johnson Co. Farm are presented in Table 2-4. Significant differences were observed for all yield and growth parameters, except percent marketability, for both years. In 2013, plants grafted with ‘Maxifort’, ‘Multifort’, ‘DRO 131’, and ‘Arnold’ rootstocks produced

significantly higher total and marketable fruit weight, number, and fruit size compared to nongrafted plants. In contrast, plants grafted with ‘RT 1028’ rootstock only increased total number of fruit compared to nongrafted plants. In 2014 plants grafted with ‘Maxifort’, ‘Arnold’, and ‘Colosus’ rootstocks produced higher total fruit weight, number, and size as well as marketable fruit weight and numbers compared to both nongrafted and self-grafted plants. Grafting plants onto ‘Colosus’ rootstock also led to increased marketable fruit size. In 2014, similar total and marketable fruit number, weight, and size was observed for plants grafted with ‘RT 1028’ and ‘RST-04-106’ rootstocks compared to nongrafted and self-grafted plants. Over the two years, compared to nongrafted plants, the average percentage of total yield increases observed for plants grafted with rootstocks: ‘Maxifort’ (65-72%); ‘Multifort’ (77%); ‘DRO 131’ (54%); ‘Arnold’ (65-66%); ‘Colosus’ (73%); ‘RST-04-106’ (18%); and ‘RT 1028’ (10-14%). In addition, compared to nongrafted plants, grafted plants increased the overall average fruit size with rootstocks: ‘Maxifort’ (24-20 g); ‘Multifort’ (27 g); ‘DRO 131’ (17 g); ‘Arnold’ (20-25 g); and ‘Colosus’ (26 g). Plant biomass compared to nongrafted plants also significantly increased for plants grafted with ‘Multifort’ and ‘Maxifort’ rootstocks in 2013 and ‘Maxifort’, ‘Arnold’, and ‘Colosus’ rootstocks in 2014.

Discussion

Over the two-year study at the four high tunnel sites, we conducted a total of eight trials with little to no disease evident. Of the seven rootstocks that were evaluated, ‘RT 1028’ and ‘RST-04-106’ rootstocks, resulted in similar yields compared to nongrafted ‘BHN 589’ plants at seven and six trials, respectively. Conversely, two rootstocks, ‘Arnold’ and ‘Maxifort’, increased

yields at four trials; and three rootstocks, ‘Multifort’, ‘DRO 131’, and ‘Colosus’, also increased yields but were only evaluated in one trial each.

Plants grafted to ‘RT 1028’ and ‘RST-04-106’ rootstocks evaluated at six and seven trials, respectively, consistently performed similar to nongrafted and self-grafted plants at all trials in regards to total fruit weight and plant biomass. However, total fruit number did increase for plants grafted onto ‘RST-04-106’ rootstock at two different trial sites in Wyandotte and Douglas counties in 2014 and for plants grafted onto ‘RT 1028’ at the Johnson County Farm in 2013, but this trend was not consistent across years. Interestingly, in the 2013 Douglas Co. on-farm trial, the plants grafted onto the ‘RST-04-106’ rootstock had reduced total fruit number as well as the fruit marketability; yet in 2014 at the same site, plants grafted onto rootstock ‘RST-04-106’ increased fruit number and the marketability was the same as nongrafted plants. Clearly, there is a need to examine data from numerous individual trials to identify consistent patterns on the productivity of particular rootstocks with various climate and farming practices. It should be noted that overall lower yields for 2014 Wyandotte Co. were most likely observed because data for the final harvest was not collected. In addition lower yields for OHREC in 2013 compared to 2014 were due to later planting dates than usual in high tunnel production.

Furthermore, our trials only tested plants grafted to ‘BHN 589’ scion and possible other scions may different results. For example, McAvoy et al. (2012) reported that in open-field environment in Virginia, ‘RST-04-106’ rootstock grafted with ‘BHN 602’ scion increased total marketable yield during trials with both high and very low disease pressure from southern bacterial wilt (*R. solanacearum*) when compared to nongrafted plants. Although selected rootstocks may be advantageous to farmers managing soilborne diseases like southern bacterial wilt, our results suggest that grafting with ‘RST-04-106’ and ‘RT 1028’ rootstocks indicate no

extra benefit, which means that these rootstocks may not be a profitable endeavor during years where environmental conditions are not suitable for disease.

‘Arnold’ and ‘Maxifort’ rootstocks were both evaluated at four trial sites each and results indicate they both may help growers increase fruit productivity in high tunnels with little to no disease pressure. Increases in total and marketable yields for both weight and number were observed for plants grafted the ‘Arnold’ rootstock compared to nongrafted plants for at least three of the four trials. The implementation of ‘Arnold’ rootstock also increased fruit size at the two Johnson Co. farm trials. Plants grafted to ‘Maxifort’ increased total yields in weight by 40% to 72% compared to nongrafts as well as increased total number and size at all four sites. Moreover, marketable yields for plants grafted with ‘Maxifort’ rootstock were significantly improved for at least three trials for each weight, number, and size parameters.

However, the growth habit of plants grafted onto ‘Arnold’ displayed less vigor in plant biomass than those grafted onto ‘Maxifort’ rootstock. In our studies, plants grafted onto ‘Arnold’ rootstock produced similar scion biomass compared to nongrafted plants in all trials except in 2014 at Johnson Co. on-farm trial where biomass increased for plants grafted to ‘Arnold’. Despite the lack of plant vigor, grafting with ‘Arnold’ rootstock increased total fruit weight by 45-66%. Similar to ‘Arnold’, grafting with rootstock ‘DRO 131’ improved both tomato total and marketable fruit yields in weight, number and size but with similar biomass production to a nongrafted plant. However, it was only examined in one trial making it difficult to draw broad conclusions. These less vigorous rootstocks that potentially increase yield may be beneficial to graft in situations where growers do not prefer a large vegetative plant.

Plants grafted onto ‘Maxifort’, on the other hand, significantly increased biomass at all four trials. Furthermore, ‘Multifort’ and ‘Colosus’ rootstocks also resulted in vigorous growth

increasing biomass and yields, but just as mentioned with rootstock ‘DRO 131’, these rootstocks were only trialed once each. The three rootstocks varieties ‘Multifort’, ‘Maxifort’, and ‘Colosus’ that displayed significantly more plant biomass than nongrafted plants, also exhibited the largest increases in fruit weight, number, and size. ‘Maxifort’ and ‘Multifort’ rootstocks are known for their vigor, which results in more plant biomass and could sustain an extended growing season (Lee, 1994; Barrett et al., 2012c; Masterson, 2013; Rivard et al., 2008). Similar to our results, Masterson (2013) found that when ‘Maxifort’ rootstock was grafted with ‘BHN 589’ tomato, both total and marketable weight and number of fruit as well as overall fruit size were significantly increased when plants were grown in high tunnels in Kansas. Moreover, in open-field trials in North Carolina, grafting with ‘Maxifort’ to heirloom ‘German Johnson’ scions increased yield 43% compared to nongrafted plants growing in soils with little to no disease pressure (Rivard et al. 2008). However, in open-field studies conducted in Washington state, Buller et al. (2013) found ‘Maxifort’ grafted with ‘Cherokee Purple’ heirloom scions did not increase fruit yield in a field with low to no disease pressure. Benefits obtainable from grafting highly depend on many factors such as rootstock and scion pairing, cultivation practices, and regional climates (Lee et al., 2010). For high tunnel growers, grafting with rootstocks bred for vigorous attributes may be the most effective for increasing the health, life span, and yield potential of a tomato crop. Commonly used in greenhouse hydroponic production, interspecific hybrid rootstocks like ‘Maxifort’ may be more popular for growers intensifying and lengthening their production in protected environments, such as high tunnels (Leonardi and Giuffrida, 2006; Masterson, 2013). Vigorous growth by the scion may be less beneficial in the open-field system where the production season is limited compared to typical greenhouse production cycles (Barrett et al., 2012c; Lee, 1994).

Grafting with select rootstocks is a tool for managing abiotic and biotic stresses such as high and low soil temperatures, soil salinity, pH, diseases, insect pest damage (Colla et al., 2010; Louws et al., 2010; Rivero et al., 2003 a and b; Schwartz et al., 2010; Savvas et. al, 2011) all of which are commonly found in high tunnels. The Central U.S. can often experience many of these stressors such as extreme weather events including early and late frost and intense summer heat. Rogers and Wszelaki (2012) speculated that extreme summer temperatures reaching up to 125 °F in high tunnels resulted in lower tomato fruit yields than expected. However, considering results from growth chamber studies by Rivero et al. (2003a), in which grafted tomato produced less of stress-related phytochemicals and more plant biomass than nongrafted plants during high growing temperatures (95 °C), grafting with selected scion and rootstock pairs may be used to manage heat stress in high tunnels.

In an evaluation of high tunnels in the Central U.S., Knewton et al. (2012) reported that high tunnels could maintain a suitable soil salinity and pH for 8 years under conventional and organic management practices. However, the EC value at the Douglas County farm (5.67 mS/cm), which had been in production three years prior to the soil test, was considered yield limiting for (nongrafted) tomato. It is not clear if the observed vigor by certain rootstocks is the result of added vigor by the rootstock or tolerance to an unidentified abiotic (or biotic) stressor such as EC; though, EC was only a factor in one trial here. Further research that investigates scion and rootstock pairings, in addition to potential physiological and genetic indicators of plant vigor and stress tolerance will be particularly valuable as it relates to the implementation of inter-specific hybrid rootstocks (Guan et al., 2012).

A primary advantage to identifying rootstocks with multiple benefits other than conferring soilborne disease resistance is that additional yield could offset the added costs of

growing or purchasing grafted plants (Rivard et al., 2008; Leonardi and Giuffrida, 2006). In cases where a rootstock provides both disease resistance and added vigor, rootstocks could then be implemented to prevent disease epidemics and potential subsequent infestation by pathogen inoculum that causes soilborne and/or root diseases. Furthermore, identifying rootstock with both characteristics provides valuable information that can be utilized by researchers and growers to predict what rootstocks will remain profitable even during years when little disease occurs due to unsuitable environmental conditions. As a result, the information provided in this report could be valuable in growing regions other than the Central U.S. However, without a clear understanding of how rootstocks function, it is hard to predict how they will perform with various scions and environments.

Future studies should not only focus on rootstock and scion selections, but also integrate high tunnel and plant management practices, especially in water and nutrient management, to optimize tomato productivity (Lee, 1994; Leonardi and Giuffrida, 2006; Djidonou et al., 2013). In open-field studies in Florida, ‘Multifort’ rootstock grafted onto ‘Florida 47’ scion was more efficient at utilizing nitrogen and irrigation applications, which resulted in increased marketable and total yields compared to nongrafted plants (Djidonou et al., 2013). It was suggested by the authors that the vigor of the rootstock and its ability to uptake nutrients and water could also require adjustments to irrigation and fertility management (Djidonou et al, 2013). Similarly, other farming practices may influence the benefit of utilizing grafted tomato in high tunnels. For example, the Johnson Co. farm trial included shade cloth and had 24” in-row spacing compared to the other 3 sites with no shade cloth and 18” spacing. Further research should focus on identifying ideal plant densities and training systems to optimize high tunnel production, especially when managing plants that have vigorous rootstocks.

The obvious cost to grafting is the additional resources required for transplant propagation or purchase (Rivard et al., 2010b; Barrett et al., 2012a). Growers need to be able to increase their productivity enough to cover the economic cost of grafting in the short-term so that long-term benefits such as reduced soilborne disease epidemics and more consistent production can be realized (Rysin et al., 2015). However, factors other than increased productivity such as management practices, grafted transplant costs, and market pricing of yield, are important to determine economic returns for grafted productions (Rysin et al., 2015). Although with significant yield improvements, such as our results with ‘Maxifort’ and ‘Arnold’ in high tunnels with no or little disease pressure, may be economically feasible even for premium grafted transplant prices, especially if selling fruit at high market price (Rysin et al., 2015).

It is important to note that although preventative use of rootstocks could help reduce the likelihood of infestation by pathogen inoculum, it does not replace crop rotation. Continuous cropping or limited crop rotation intervals may cause pathogen resistance to effective rootstocks (King et al., 2008). Growers should continue to use integrated pest management with crop and rootstock rotations to help prevent persistent pest and disease issues (King et al., 2008).

Conclusion

Increasing tomato productivity with grafting can increase the efficiency of limited space and tolerance of stressful conditions often associated in high tunnel production. Our results indicate that grafting with ‘Arnold’ and ‘Maxifort’ rootstocks is advantageous for urban and peri-urban high tunnel tomato growers in the Central U.S., and significant effects were seen related to marketable and/or total yield in all four of their trial site years for both rootstocks. Meanwhile, grafting with ‘RST-04-106’ and ‘RT 1028’ rootstocks conferred no benefit under

little disease pressure in all of their seven and six trial site years, respectively. It should be noted that these trials were conducted with a single scion, 'BHN 589', in order to provide consistent data and experience with other rootstock and/or scion combinations are needed to verify this trend. However, this data can be combined with plant disease trial data from other locations to ascertain the benefits of grafting with select rootstocks during years that may not experience significant disease. Furthermore, this report provides information for growers related to rootstocks that may not be beneficial in the region. To our knowledge, this is the first report of rootstocks that consistently performed poorly in high tunnel trials where no disease pressure is evident. However, our trials only paired rootstocks with one scion; therefore, clearly further research is needed to identify ideal tomato rootstock and scion combinations as well as management

Table 2-1: Tomato fruit yield and plant biomass of nongrafted and grafted plants at the Olathe Horticulture Research and Extension Center high tunnel site in 2013 and 2014

Treatment	Marketable Fruit Yield ^x			Total Fruit Yield			% Marketability ^v		Biomass ^u
	Weight ^s (kg/plant)	Number ^r (fruit/plant)	Size (g) ^w	Weight (kg/plant)	Number (fruit/plant)	Size (g)	Weight	Number	Dry Weight (g/plant)
<i>2013^y</i>									
Nongrafted ^z	6.5	30	215	7.4	35	212	89	87	341
Self-grafted	6.4	32	204	7.5	37	202	86	85	387
RT 1028	6.6	31	212	7.6	37	208	87	86	521
RST-04-106	6.8	33	209	7.9	38	206	87	86	345
<i>2014</i>									
Nongrafted	15.1 b ^t	70 b	215 bc	16.5 b	78 b	211 c	92	90	387 b
Self-grafted	14.9 b	69 b	216 bc	16.2 b	76 b	215 bc	92	91	351 b
Maxifort	21.0 a	91 a	230 a	23.1 a	101 a	230 a	91	91	494 a
RT 1028	14.9 b	70 b	212 c	16.3 b	77 b	212 c	91	91	396 b
RST-04-106	14.6 b	66 b	221 b	16.3 b	75 b	219 b	89	88	393 b

^z Nongrafted and self-grafted (plants grafted onto its own root system) are tomato cv. BHN 589. Other treatments indicate the rootstock var. that is grafted to cv. BHN 589 scion.

^y Experiment was arranged in a RCBD with each of the 6 blocks in six identical high tunnels (1 rep per tunnel). In each plot, 9 and 6 plants were planted 18" apart in 2013 and 2014, respectively. Planting dates were 31 May, 2013 and 21 Apr., 2014.

^x Fruit was harvested 1-2 times per week and graded as marketable or unmarketable base on fruit issues such as pest damage, fruit cracks, and blossom-end rot. At the end of trial all fruit larger than 5 mm was counted and weighed.

^w Average fruit size was determined by dividing number of fruit by fruit weight.

^v Percent marketability was determined by dividing marketable yield by the corresponding total yield measurement (weight or number).

^u Biomass includes whole scion plant sample harvested at end of season and dried at 70 °C for at least 72 hrs.

^t Different letters show significant differences between values. Yield measurements were analyzed independently each year.

^s Marketable and total fruit weight and size, % marketability by weight and dry biomass were analyzed using Fisher's protected LSD (P <0.05).

^r Marketable and total fruit count and % marketability by number was analyzed using Poisson model ($\alpha = 0.05$).

Table 2-2: Tomato fruit yield and plant biomass of grafted and nongrafted at Wyandotte County, KS high tunnel site in 2013 and 2014

Treatment	Marketable Fruit Yield ^x			Total Fruit Yield			% Marketability ^v		Biomass ^u
	Weight ^s (kg/plant)	Number ^r (fruit/plant)	Size (g) ^w	Weight (kg/plant)	Number (fruit/plant)	Size (g)	Weight	Number	Dry Weight (g/plant)
<i>2013^y</i>									
Nongrafted ^z	6.3	49 a ^t	128	7.4	61	122	85 ab	82	290
Self-grafted	5.2	40 ab	131	6.4	51	124	81 bc	77	221
RT 1028	5.4	38 b	143	6.6	49	135	80 c	75	310
RST-04-106	5.8	43 ab	134	6.7	53	127	86 a	82	313
<i>2014</i>									
Nongrafted	3.5 ab	21 b	167	3.7 ab	23 b	161	94	92	100
Self-grafted	3.0 b	21 b	147	3.3 b	23 b	143	92	89	126
Arnold	5.6 a	34 a	165	6.1 a	37 a	163	90	90	173
RST-04-106	4.3 ab	30 a	141	4.7 ab	35 a	136	91	88	166

^z Nongrafted and self-grafted (plants grafted onto its own root system) are tomato cv. BHN 589. Other treatments indicate the rootstock var. that is grafted to cv. BHN 589 scion.

^y Experiment was arranged in a RCBD with 4 blocks at the ends of two high tunnels (1 block at each end) in 2013 and within 2 rows of one high tunnel (2 blocks per row) in 2014. Within each treatment plot, 6 plants were planted 18" apart. Planting dates were 12 Apr., 2013 and 7 Apr., 2014.

^x Fruit was harvested 1-2 times per week and graded as marketable or unmarketable base on fruit issues such as pest damage, fruit cracks, and blossom-end rot. At the end of trial in 2013 all fruit larger than 5 mm was counted and weighed, but 2014, trial ended without a final harvest.

^w Average fruit size was determined by dividing number of fruit by fruit weight.

^v Percent marketability was determined by dividing marketable yield (weight or number) by the corresponding total yield measurement (weight or number).

^u Biomass includes whole scion plant sample harvested at end of season and dried at 70 °C for at least 72 hrs.

^t Different letters show significant differences between values. Yield measurements were analyzed independently each year.

^s Marketable and total fruit weight and size, % marketability by weight and dry biomass were analyzed using Fisher's protected LSD (P <0.05) .

^r Marketable and total fruit count and % marketability by number was analyzed using Poisson model ($\alpha = 0.05$).

Table 2-3: Tomato fruit yield and plant biomass of nongrafted and grafted plants at the Douglas County, KS high tunnel site in 2013 and 2014

Treatment	Marketable Fruit Yield ^x			Total Fruit Yield			% Marketability ^v		Biomass ^u
	Weight ^s (kg/plant)	Number ^r (fruit/plant)	Size (g) ^w	Weight (kg/plant)	Number (fruit/plant)	Size (g)	Weight	Number	Dry Weight (g/plant)
<i>2013^y</i>									
Nongrafted ^z	6.2 a ^t	40 ab	159 a	8.5	58 a	147	74 a	69	267
Self-grafted	5.7 ab	42 a	137 b	7.9	57 a	138	70 ab	71	264
RT 1028	4.3 b	30 bc	141 ab	6.3	47 ab	135	67 ab	65	262
RST-04-106	4.1 b	28 c	145 ab	6.5	45 b	144	61 b	60	331
<i>2014</i>									
Nongrafted	7.1 b	44	162 abc	8.8 b	54 b	163 abc	81	81 a	205 b
Self-grafted	9.1 ab	58	158 bc	11.3 ab	72 a	157 bc	81	80 a	205 b
Arnold	9.9 a	59	168 ab	12.7 a	76 a	167 ab	79	78 a	288 ab
RST-04-106	8.7 ab	58	149 c	11.1 ab	75 a	147 c	79	78 a	271 ab
Maxifort	10.5 a	58	181 a	13.9 a	77 a	180 a	76	75 b	371 a

^z Nongrafted and self-grafted (plants grafted onto its own root system) are tomato cv. BHN 589. Other treatments indicate the rootstock var. that is grafted to cv. BHN 589 scion.

^y Experiment was arranged in a RCBD with 4 blocks within 2 rows inside one high tunnel (2 blocks per row). Within each treatment plot, 6 plants were planted 18" apart. Planting dates were 23 Apr., 2013 and 11 Apr., 2014.

^x Fruit was harvested 1-2 times per week and graded as marketable or unmarketable base on fruit issues such as pest damage, fruit cracks, and blossom-end rot. At the end of trial all fruit larger than 5 mm was counted and weighed.

^w Average fruit size was determined by dividing number of fruit by fruit weight.

^v Percent marketability was determined by dividing marketable yield (weight or number) by the corresponding total yield measurement (weight or number).

^u Biomass includes whole scion plant sample harvested at end of season and dried at 70 °C for at least 72 hrs.

^t Different letters show significant differences between values. Yield measurements were analyzed independently each year.

^s Marketable and total fruit weight and size, % marketability by weight and dry biomass were analyzed using Fisher's protected LSD (P <0.05).

^r Marketable and total fruit count and % marketability by number was analyzed using Poisson model ($\alpha = 0.05$).

Table 2-4: Tomato fruit yield and plant biomass of grafted and nongrafted at Johnson County, KS high tunnel trial site in 2013 and 2014

Treatment	Marketable Fruit Yield ^x			Total Fruit Yield			% Marketability ^v		Biomass ^u
	Weight ^s (kg/plant)	Number ^r (fruit/plant)	Size (g) ^w	Weight (kg/plant)	Number (fruit/plant)	Size (g)	Weight*	Number	Dry Weight (g/plant)
<i>2013^y</i>									
Nongrafted ^z	7.8 c ^t	56.7 c	138 b	8.1 c	60.0 c	135 b	97	94	257 c
Maxifort	13.4 ab	81.8 a	163 a	13.9 ab	89.6 ab	155 a	96	91	1089 ab
Multifort	13.7 a	82.6 a	166 a	14.3 a	88.6 a	162 a	96	93	1634 a
DRO 131	11.9 b	74.8 ab	160 a	12.5 b	82.4 ab	152 a	96	91	756 bc
Arnold	12.8 ab	80.3 a	160 a	13.4 ab	85.9 ab	155 a	96	93	768 bc
RT 1028	8.8 c	66.9 bc	131 b	9.2 c	73.5 b	126 b	95	91	415 c
<i>2014</i>									
Nongrafted	9.4 b	62.9 b	150 b	9.9 b	68.2 b	145 b	95	92	211 c
Self-grafted	9.3 b	63.3 b	147 b	9.6 b	65.9 b	146 b	97	96	151 c
Maxifort	15.6 a	90.7 a	172 ab	16.3 a	96.5 a	169 a	95	94	475 a
Arnold	15.7 a	91.3 a	172 ab	16.4 a	96.6 a	170 a	96	94	327 b
RST-04-106	11.3 b	71.1 b	159 ab	11.7 b	75.0 b	156 ab	97	95	240 bc
RT 1028	10.5 b	67.2 b	156 ab	10.9 b	72.1 b	151 ab	96	93	244 bc
Colosus	16.6 a	95.9 a	173 a	17.1 a	99.9 a	171 a	97	96	453 a

^z Nongrafted and self-grafted (plants grafted onto its own root system) are tomato cv. BHN 589. Other treatments indicate the rootstock var. that is grafted to cv. BHN 589 scion.

^y Experiment was arranged in a RCBD with 4 blocks within in the same high tunnel (1 block per row). Within each treatment plot, 6 plants were planted 24" apart. Planting dates were 5 Apr., 2013 and 2 Apr., 2014.

^x Fruit was harvested 1-2 times per week and graded as marketable or unmarketable base on fruit issues such as pest damage, fruit cracks, and blossom-end rot. At the end of trial all fruit larger than 5 mm was counted and weighed.

^w Average fruit size was determined by dividing number of fruit by fruit weight.

^v Percent marketability was determined by dividing marketable yield (weight or number) by the corresponding total yield measurement (weight or number).

^u Biomass includes whole scion plant sample harvested at end of season and dried at 70 °C for at least 72 hrs.

^t Different letters show significant differences between values. Yield measurements were analyzed independently each year.

^s Marketable and total fruit weight and size, % marketability by weight and dry biomass were analyzed using Fisher's protected LSD (P <0.05).

^r Marketable and total fruit count and % marketability by number was analyzed using Poisson model ($\alpha = 0.05$).

Chapter 3 - The Effect of Leaf removal on Adventitious Root Formation and Plant Growth of Grafted Tomatoes

Introduction

Grafted Solanaceae and Cucurbitaceae vegetable species are used worldwide for managing abiotic and biotic stresses and particularly in intensively-cultivated production systems such as: greenhouses, high tunnels, small farms, and urban agricultural settings (Kubota et al., 2008; Lee, 1994; Louws et al., 2010; Guan et al., 2012). Many vegetable farmers in the United States are interested in using grafted vegetable plants for commercial production, but have limited purchasing options or ability to graft their own plants (Kubota et al., 2008; Rivard et al., 2010b). We surveyed fruit and vegetable growers (n=265) at the 2014 Great Plain Growers Conference, in St. Joseph, MO and they reported that 19% are using grafted vegetables but an additional 56% are interested in learning more or incorporating grafted plants in their production (Rivard, unpublished data). Furthermore, 47% would prefer to graft their own while 25% would rather purchase plants (Rivard, unpublished data). On-farm grafted propagation (as opposed to purchasing grafted plants) is often preferred for many small-scale growers as they can match particular combinations of rootstock and scion cultivars in order to overcome site-specific issues to while catering to specialty or niche markets (Rivard et al., 2010). Tomato (*Solanum lycopersicum*) is relatively simple to graft compared to other commonly grafted vegetables such as pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.) and watermelon (*Citrullus lanstus*) (Kubota et al., 2008), and is the most popular vegetable grown in high tunnels in the United States (Carey et al., 2009). Small-acreage growers, especially in urban and peri-urban areas, typically plant small batches of

various cultivars, particularly heirloom varieties, for specialty markets, which also contribute to the difficulty of purchasing grafted plants. For tomato, heirloom cultivars, which normally may not have disease resistance or production characteristics of modern hybrids, benefit from grafting and may be a particular reason why small-acreage growers are among the most interested in using the technique (Barrett et al., 2012c; Rivard and Louws, 2008). Purchasing options for commercially grafted tomatoes in the U.S. is limited to a few propagation companies in the east, west, and southwest U.S. but are also available for import from Canada and Mexico. However, shipping transplants long distances and/or across borders causes alarm for transplant quality deterioration and possible seed contamination and disease epidemic (Kubota et al., 2008).

Although small-acreage vegetable growers could benefit from grafting their own plants, many that are new to grafting and/or inexperienced with plant propagation may have difficulties producing high quality, grafted tomato transplants. Ideally, growers want a tomato transplant with good root growth and compact foliage growth that is supported by a thick stem and strong graft (Vu et al., 2013). Along with strong vascular connection, a high quality grafted tomato has an insignificant amount limited adventitious roots (AR) from the scion (Bumgarner and Kleinhenz, 2014; Lee, 1994; Rivard and Louws, 2011). If allowed to grow into the soil/media, AR from the scion can reduce grafted transplant quality by decreasing rootstock growth and function, sometimes leading to graft failure and rootstock death. For a grower utilizing grafting for soilborne disease management, AR formation may increase the chances of disease in the production field by exposing the susceptible scion tissue to pathogen-infested soils (Lee et al., 2010; Rivard and Louws, 2011).

Splice grafting, also called tube grafting, is the most commonly-used grafting method for tomato due to its relatively low cost, as well as its effectiveness at producing high quality grafted transplants (Kubota et al., 2008; Oda, 1999; Rivard and Louws, 2011). However, because the root system is completely removed from the scion during grafting, post-grafting environmental management is critical so that the scion tissue stays alive while the vascular system reconnects to the new rootstock. Therefore, grafted plants are immediately placed in a healing chamber; an environment with high humidity and low light used to prevent excessive water stress on the scion tissue while the graft union heals (Bumgarner and Kleinhenz, 2014; Kubota et al., 2008; Oda, 1999; Rivard and Louws, 2011). It typically takes at least 4 days for vascular connection to begin and 15 days for complete repair and callus formation (Fernández-García et al., 2004a). Propagators are recommended to keep plants in the healing chamber for 7 to 10 days (Kubota et al., 2008; Oda, 1999; Rivard and Louws, 2011; Vu et al., 2013).

Adventitious root formation in plants is not completely understood and many exogenous factors, such as nutrition, humidity, light, temperature, and surrounding biota, in addition to endogenous factors, such as aging, phytohormones and other phytochemicals, can influence the promotion and inhibition of AR (Geiss et al., 2010). Vegetable grafting extension publications mention AR formation and speculate that it occurs due to an excessive time in the high humidity environment post-grafting (Johnson et al., 2011).

Recent research reported high success rates of grafted tomato seedlings that were healed in lower humidity chambers with shade cloth alone, with no plastic enclosure, and average relative humidity (RH) ranged from 53% to 69% (Johnson and Miles, 2011; Masterson et al., in press). In a controlled environment study by Vu et al. (2013), optimal conditions to maximize grafted tomato plant survival rates (100%) included 90% RH in healing chambers for the first 2-

3 days and 70% for the following 7-8 days. Furthermore, on day 10 post-grafting, Vu et al. (2013) compared the effect of humidity on transplant quality of grafted plants that were healed in environments with different levels of RH by comparing different parameters: percent of diseased plants, plant height, stem diameter, number of leaves, leaf chlorophyll content, leaf area, root and shoot dry biomass, shoot to root ratio, and plant compactness. Plants that healed in chambers with lower (70%) RH for 10 days were shorter and had less biomass than the ones that were healed in the high humidity chamber (90%) for 10 days. The low humidity chamber, however, showed similar transplant quality to plants in the optimal conditions described above. However, both the low humidity chamber (70%) and 10-day high humidity chamber (90%) produced less compact plants than plants from the optimal conditions (Vu et al., 2013).

Furthermore, Masterson et al. (in press) reported that removing scion leaves increased graft survival rates. Leaf removal of the scion is a technique used to help plant tolerate water stress post-grafting and increase grafting success (Bumgarner and Kleinhenz, 2014; Masterson et al., in press). The act of grafting and removing scion leaves could invoke a plant response to wounding and/or water stress, and AR formation during the graft healing process could be impacted by this procedure as well (Guan et al., 2012).

The process of cutting the scion completely from its rootstock during splice grafting is similar to that of vegetative propagation, in which a young shoot is excised and AR is promoted. Indeed, much of the literature about AR is related to vegetative propagation, in which AR is a desired process. Extensive research observing AR formation has reported that the plant hormone auxin, which is produced in the shoot meristem young leaves and cotyledons, travels basipetally and accumulates at the wounding site of vegetatively-propagated plants (Katsumi et al., 1969; Maldiney et al., 1986; Nordström and Eliassum, 1991). Among the many functions of auxin is its

promotion of xylem tissue regeneration and AR formation. Propagators wanting to promote AR will immediately apply auxin exogenously to the severed end of a plant cutting. (da Costa et al., 2013; Kevers et al., 1997). High auxin to cytokinin ratio in plant tissues is associated with AR formation (da Costa et al., 2013; Kevers et al., 1997; Katsumi et al., 1969; Maldiney et al., 1986; Nordström and Eliassum, 1991). Other plant hormones such as ethylene, abscisic acid, and jasmonic acid are known to cross-talk with auxin to either promote or inhibit AR (da Costa et al., 2013). Although a complete understanding of all hormones and other phytochemicals associated with AR formation is still unclear, there is ample evidence that auxin is involved (da Costa et al., 2013). Removing leaves may have an indirect impact affect in AR formation, as auxin production is located in young leaves (Katsumi et al., 1969; Maldiney et al., 1986; Nordström and Eliassum, 1991).

In addition to leaf removal effect on the formation of AR, the loss of photosynthesizing area and carbohydrate production may also influence transplant quality, growth, and maturity. Bumgarner and Kleinhenz (2014) discuss using leaf removal for tomato grafting to decrease transpiration, but also note that it would increase wounding sites open for possible disease contamination as well as possibly reduce photosynthesis and carbohydrate production during the 3-week post-grafting acclimation period. Following acclimation, propagators will want the highest quality grafted transplant.

Leaf removal could be a valuable technique for small-scale propagators that are wishing to graft their own tomato plants, but it is not clear how this practice affects the transplant quality and early plant growth of grafted plants. It is important for propagators and farmers to understand how grafting techniques may also affect plant quality to justify application. Moreover, leaf removal could impact AR formation as the young leaves provide the plant

hormone auxin, which is associated with AR formation (Katsumi et al., 1969; Maldiney et al., 1986; Nordström and Eliassum, 1991). Therefore, the objectives of the studies in this report were to: (i) investigate how scion leaf removal affects the formation of adventitious roots, (ii) determine the impact of scion leaf removal on grafted transplant quality and (iii) identify the effect of scion leaf removal on early plant growth and development post-grafting.

Materials and Methods

In order to address our three research objectives, two different, but complementary greenhouse studies were conducted during Spring 2014 at the Kansas State University Olathe Horticulture Research and Extension Center (OHREC). The goal of the first study was to identify the effect of scion leaf removal on AR formation (objective 1) and its influence on transplant quality (objective 2). The aim of the second study was to measure plant growth parameters and further develop information on the impact of leaf removal on transplant quality (objective 2) and plant quality in the first 3 weeks after transplant (objective 3). All experiments were conducted in a Quonset-style greenhouse that had 10-mm twin-wall polycarbonate walls and a typical commercial greenhouse double-layer, 6-mil polyethylene film roof. Popular hybrid tomato cv. ‘BHN 589’ (BHN Seed; Immokalee, FL) often recommended for high tunnel production was used as the scion and commonly used vigorous tomato rootstock cv. ‘Maxifort’ (De Ruiter; St. Louis, MO) was selected as the rootstock. Both scion and rootstock were sown in Fafard Germination Mix media (Conrad Fafard Inc.; Agawam, MA) on 19 March and 21 March, respectively, to accommodate for the variable germination speed of rootstock and scion cultivars. On 1 April, seedlings were transplanted into 50-cell propagation trays using Fafard 3B Mix (Sun Gro Hort Canada Ltd.; Seba Beach, AB Canada). The plants were allowed to grow under

standard greenhouse conditions for 12-14 days after transplanting prior to being grafted. This represents the time that it took for the rootstock and scion to grow to the appropriate stem diameter for splice grafting. During the course of the experiments, grafted transplants were exposed to two different greenhouse environments. Standard greenhouse environmental conditions ranged from 82 °F (day) to 64 °F (night) and plants were fertilized weekly with an application of Peter's Excel 15-5-15 Cal-Mag (Everris NA Inc.; Dublin, OH) water soluble fertilizer at a rate of 150 ppm N. Standard greenhouse environmental conditions were utilized for seed germination, transplant production, and post-grafting growth. When plants were moved into the healing chamber, the environmental conditions within the greenhouse changed to range from 76 °F to 64 °F (day) and 82 °F to 70 °F (night). Plants were not fertilized while in the healing chambers. Nongrafted plants were kept in a cool greenhouse with temperature set at 55 °F to 85 °F to slow plant growth while grafted plants healed in the chamber for 10 days. Following 10 days in the chamber, grafted and nongrafted plants were placed back in standard growing conditions for the remaining time of the experiments.

Adventitious Root Formation Study

Three levels of scion leaf removal were observed for post-grafting AR formation and growth. The three leaf removal treatments tested were 0%, 50%, and 90% true leaf removal (Fig. 3-1A). Cotyledons were left intact and attached which is the preferred method by grafters (Bumgarner and Kleinhenz, 2014). The 0% leaf removal method tested here is the standard splice grafting method, which served as the standard control. Plants that underwent 50% and 90% leaf removal had a portion of their leaves removed from 'BHN 589' scion, and were trimmed using florist's scissors. Leaves were removed immediately prior to grafting with care not to disturb the apical meristem or cotyledons. Percentage of leaf removal was determined and

cut by one person to help control for any biased treatment application. The scion was then grafted onto 'Maxifort' rootstock using the splice grafting method (Rivard and Louws, 2011) with each individual grafter responsible for complete replicate blocks in order to prevent any grafting bias. Grafted plants were placed in a healing chamber arranged in a randomized complete block design with 4 blocks and 20 plants for each experimental unit (Fig. 3-1B). The experiment was conducted in three different chambers: in a shade chamber, a plastic-enclosed chamber, and a humidified chamber (described below).

Healing Chambers

The experiment was conducted in three different healing chambers in order to make general observations about different types of chambers that may be utilized by propagators. In each experimental run, the treatments were replicated independently within each chamber (n=4). The three chamber designs were similar to Masterson et al. (in press) and included the following: shade cloth only (shade); shade cloth and polyethylene film (plastic); and shade cloth, polyethylene film, and a cool-mist humidifier (humidifier) (Fig. 3-1C). The chambers were constructed with 1" x 6" plastic lumber into a 4' x 3' x 6" base frame. Holes were drilled into the top (1" edge) of the plastic boards, and three 9-gauge wires, approximately 24" apart were inserted into the holes and hooped over the frame creating a chamber that was approximately 18" in height. Both plastic and humidifier chambers were enclosed using 4 mil clear plastic sheeting and included three, 4"-diameter openings on top to prevent excess heat build-up. The humidifier chamber had the same design as the plastic chamber except that a cool-mist humidifier (SU-2000, Sunpentown, City of Industry, CA) that delivered vaporized water through a 2" polyvinylchloride (PVC) pipe to the enclosed chamber. Three removable layers of 50% shade cloth (SunBlocker Premium, Growers Supply; Dyersville, IA) were placed on top of each

chamber and one layer of 50% shade cloth was fixed to the rafters of the greenhouse. Standard nursery (web) trays were turned upside down and placed on the bottom of the plastic and humidifier chamber to hold grafted plant trays out of standing water on the floor of the chambers. Fresh water was added in the bottom of plastic and humidifier. Additionally, trays of water were placed beneath grafted plants, in shade chambers for up to 12 hours for the first 3 days in order to increase humidity passively.

Temperature and RH within the healing chambers was recorded using a data logger (EL-USB-2-LCD, Lascar Electronics, Erie, PA) placed in the middle of the shade, poly, and humidifier chambers. Although this data was not replicated, it provides descriptive information about the microclimate within each chamber. During the first 7 days post-grafting, the average temperature and RH in the shade, plastic, and humidifier chambers were 67.6 °F and 68%; 70.9 °F and 95%; and 71.8 °F and 95%, respectively.

In order to distribute the labor required for grafting and subsequent AR ratings, the three experiments were initiated (grafted) one day apart. Therefore, experimental plants were grafted on 13 April, 14 April, and 15 April and immediately placed into the shade, plastic, and humidifier chambers, respectively. After 3 days of healing at low light, plants were slowly acclimated to higher light by removing layers of shade cloth; shade cloth was left on during sunny days. By day 7, all shade cloth was removed from chambers and polyethylene film sides were lifted from plastic and humidifier chambers to allow the relative humidity to decrease. On day 8, the polyethylene film was completely removed from the plastic and humidifier chambers. For the humidifier chamber, the cool-mist humidifier was set to high to increase humidity quickly in the chamber during the first day, but was turned down to low on day 2 and was turned

off on day 7. Plants were moved to full light and standard greenhouse environmental conditions on day 10.

Post-Grafting Adventitious Root Rating

On days 10, 17, and 24 days post-grafting, all plants were rated for AR formation on the scion using the rating system presented in Fig 3-2. The AR rating scale was developed based on preliminary experiments with grafted transplants. The ratings start at zero, indicating no AR formation or root initials observed on the epidermis. When root initials were observed through the epidermis of the scion, plants were determined to have a single or multiple amount of trace roots (< 2 mm), small roots (≥ 2 mm), medium root (≥ 5 mm) and/or large roots (≥ 10 mm), which determined their AR rating from a scale of 0 to 10 (0 = none; 1 = trace root(s) only; 2 = ≥ 1 small root(s) and any trace root(s); 3 = 1 medium root and any small or trace root(s); 4 = multiple medium roots and any small or trace root(s); 5 = 1 large root and any additional roots but none in the soil; 6 = multiple large roots and any additional roots but none in soil; 7 = 1 root in soil and any small or trace root(s); 8 = 1 root in soil and any medium or large root(s); 9 = multiple roots in soil and any additional roots; 10 = multiple roots in soil causing promotion of scion growth by AR and failure of rootstock growth) (Fig 3-2). Up to an AR rating of 6, none of the AR had made contact with the soil/media. Soil contact further increased ratings from 7 to 10, in which the graft was no longer functioning. During data analysis, the numerical ratings were grouped into three categories (acceptable, manageable, and unacceptable), based on the overall effect of AR formation on transplant quality and marketability. Ratings 0-3 had insignificant adventitious rooting and considered acceptable for use. Ratings 4-6 were categorized as manageable because most likely a grower producing their own transplants could trim the roots before planting to ensure no AR soil contact. If AR had made contact with the soil, plants had

ratings of 7-10 and were considered unacceptable. These unacceptable ratings represent an unmarketable plant for a propagator, and also unusable to a grower producing their own transplants. Twenty-four days after grafting, all AR were removed from the scion and the fresh weight of AR was recorded.

Transplant Quality and Plant Growth Study

Plants for the second study were seeded, grafted, and given post-grafting care identical to the shade chamber experiment mentioned in previous section. Following the first experiment, the plants from the shade chamber had very little to no AR formation and resulted in no unacceptable grafted transplants. Therefore, we continued to use these plants for the second experiment measuring plant growth parameters. Though the objective of the study was to compare leaf removal options for growers opting to use grafted plants, nongrafted var. BHN 589 tomato were incorporated into the experimental design as an additional comparison. Nongrafted plants were also seeded on the same day (19 March), grown in the same manner as the grafted plants except they were placed in a cool greenhouse (55 °F to 85 °F) for 10 days while the grafted plants were in the healing chamber. Each of the experimental units was comprised of 15 plants and was arranged as a randomized complete block design with 4 blocks.

Grafted and nongrafted plants (10-mm soil plugs) were transplanted into 4" pots on 8 May (26 days post-grafting) and then transplanted into 2.5 gal containers on day 35. Plants were destructively sampled on days 24, 31, 38, 45, and 52 post-grafting in order to observe plant growth. At each sampling date, three random plants per experimental unit were measured for leaf area, shoot biomass, root biomass, plant height, stem caliper, and flower count. Plants were severed at the soil level and plant height was measured to the apical meristem. Stem caliper measurements were taken 1 cm above the scion cotyledons using a 147 Digital Fractional Caliper

(General Tools and Instruments LLC; New York, NY). Leaf area was measured with a LI-3100 Area Meter (Li-cor Inc., Lincoln, NE). All leaf and stem, and flower tissue was combined, dried at 158 °F for at least 72 hours, and weighed so as to measure shoot biomass. Plant roots were carefully washed to remove debris, dried and weighed in the same manner. Flowers were categorized into three stages: buds (still closed), yellow (showing any sign of yellow), and pollinated (swelled ovary).

It takes approximately 21 days post-grafting for a grafted tomato seedling to completely heal and be ready for transplant or sale (Bumgarner and Kleinhenz, 2014; Rivard et al., 2010b). Therefore, the measurements taken 24 and 31 days post-grafting are the most indicative of the ideal plant size for a propagator that wishes to plant or sell grafted transplants, while day 38, 45, and 52 post-grafting represented tomato early growth and development.

Data Analysis

Adventitious rooting was quantified on days 10, 17, and 24 post-grafting. All data were analyzed using PROC GLIMMIX (SAS 9.2; SAS Institute, Cary, NC USA). Least significant differences for post-grafting AR ratings on days 7, 17, and 24, as well as the fresh weight of AR excised from scion on day 24, were analyzed independently and compared with Tukey's method where $\alpha=0.05$.

Although destructive sampling was utilized, the transplant and early growth experiment was dependent on time within a shared (greenhouse) environment. Therefore, PROC GLIMMIX (SAS 9.2; SAS Institute, Cary, NC USA) was used to analyze stem diameter, height, leaf area, root and shoot biomass, root and shoot ratio, compactness, and flower counts separately on days 24, 31, 39, 45, and 52 post-grafting. Growth parameters at days 24 and 31 after grafting were considered for the transplant quality assessments. Growth parameters at days 38, 45, and 52 days

after grafting were considered during the “early growth” assessments. Plant compactness was calculated by dividing the shoot biomass by plant height. Based on the residuals of each data set, the best model for covariance structure was selected for each parameter. For stem caliper and plant height, we used first-order autoregressive. For leaf area, root biomass, flower count, and compactness, we used heterogeneous compound symmetry. For shoot biomass and shoot-to-root ratio, we used heterogeneous first-order autoregressive. Furthermore, flower count data y , which included several 0 count data, was transformed where $y^* = \sqrt{y + \frac{3}{8}}$ for analysis using the Poisson model. Furthermore, we used the Bonferroni method to compare treatment LSDs for each independent growth parameter separately on each sampling day with $\alpha=0.05$.

Results

Effect of Leaf Removal on Adventitious Root Formation

Grafted plant survival for all three chamber environments (shade, plastic, humidifier) ranged from 95%-100% across all leaf removal treatments (data not shown). In the shade chamber, both 50% and 90% leaf removal significantly reduced the average AR rating and AR root mass compared to the 0% leaf removal ($\alpha=0.05$; Table 3-1). However, in the plastic and humidifier chambers, only the plants that underwent the 90% leaf removal method had significantly decreased AR ratings and AR fresh weight, while the 50% leaf removal method resulted in statistically similar levels of AR development compared to the standard method of 0% leaf removal ($\alpha=0.05$; Table 3-1).

Effect of Leaf Removal on Transplant Quality

Transplant quality for days 24 and 31 post-grafting was determined by the AR formation ratings in the first experiment and growth parameters (stem diameter, height, shoot and root biomass, and compactness) in the second experiment. When comparing to the standard method of 0% leaf removal, removing 90% of scion leaves decreased unacceptable levels of AR in all three chamber environments but only in the shade chamber for 50% leaf removal (Figure 3-3). Furthermore, post-grafting growth measurements for plants from the shade chamber indicate that plants with 90% leaf removal method decreased transplant quality in leaf area, shoot biomass, root biomass, and compactness, but 50% leaf removal method had similar transplant quality when compared to 0% leaf removal method except for root biomass on day 24 and leaf area on day 31 (Table 3-2; Figure 3-4).

In the shade chamber on day 24 after grafting, 97% and 99% of the 50% leaf removal and 90% leaf removal grafted plants, respectively, were considered acceptable, 1-3% were considered manageable, and 0% were considered unacceptable (Fig. 3-3; A and B). In contrast, the standard method (0% leaf removal) had 71% acceptable, 19% manageable, and 10% unacceptable grafted transplants from the shade chamber (Fig. 3-3A). For the plastic and humidifier chambers, 90% leaf removal also had high acceptable rates of 98% and 95%, respectively, and 0% unacceptable grafts (Figures 3-3B and C). However, plants with 50% leaf removal in the plastic and humidifier chambers had acceptable, manageable, unacceptable rates of 65%, 26%, 9% and 60%, 31%, 9%, respectively (Fig. 3-3; B and C). The standard method of 0% leaf removal resulted in the lowest proportion of acceptable ratings in the plastic and humidifier chambers at 42% and 50%, as well as the highest percentage of manageable rates of 42% and 50% and unacceptable rates of 8% and 17% (Figures 3-1B and C).

Root biomass was significantly less for 50% leaf removal than 0% leaf removal on day 24 ($\alpha=0.05$; Table 3-2), but on day 31, the two methods showed similar root biomass as well as similarities in all other growth parameters used to measure transplant quality (Table 3-2; Figure 3-4). Interestingly, root biomass for 90% leaf removal produced opposite results with similar root biomass as 0% leaf removal on day 24, but significantly less root biomass than 0% leaf removal on day 31 ($\alpha=0.05$; Table 3-2; Figure 3-4E). Both transplant height and stem diameters were similar among all three grafted treatments (0%, 50%, and 90% leaf removal) (Table 3-2; Figure 3-4A and B).

Using the standard technique of 0% leaf removal, grafted transplants with ‘BHN 589’ scion and ‘Maxifort’ rootstock produced significantly less shoot biomass than nongrafted ‘BHN 589’ transplants on both day 24 and 31 as well as smaller stem caliper and compactness on day 31 ($\alpha=0.05$; Table 3-2; Figure 3-4A, C and F). Furthermore when comparing to nongrafted transplants, grafted plants with 50% leaf removal decreased stem diameter, shoot and root biomass and compactness and grafted transplants with 90% leaf removal decreased all the transplant quality growth measurements: stem diameter, height, leaf area, shoot and root biomass, and compactness ($\alpha=0.05$; Table 3-2; Figure 3-4).

Effect of Leaf Removal on Early Plant Growth and Development

The effect of leaf removal on early plant growth and development for grafted transplants was determined by comparing growth measurements and flower counts on day 38, 45, and 52 for plants with 50% or 90% leaf removal to plants with 0% leaf removal (standard method of grafting). Results can be seen in Table 3-2 and Figures 3-4 and 3-5. Although the compactness of all grafted plants were similar, using the method 90% leaf removal of scion reduced stem caliber, height, leaf area, and shoot and root biomass (Table 3-2; Figure 3-4). In contrast, grafted plants

with 50% leaf removal showed no effect on early plant growth compared to standard grafting method (0% leaf removal). During sampling for early growth, grafted plants were seen to surpass nongrafted plants in leaf area (day 38, 45, and 52), shoot (day 52) and root biomass (day 45), and compactness (day 45) (Table 3-2; Figure 3-4C, D, E, and F).

Stem diameter was significantly smaller for grafted plants with 90% leaf removal for days 38, 45, and 52 when compared to both 0% leaf removal as well as nongrafted plants ($\alpha=0.05$; Table 3-2; Figure 3-4A). The 50% leaf removal method resulted in consistently similar stem diameters to both 0% leaf removal and 90% leaf the sampling dates (Table 3-3; Figure 3-4A). Furthermore, the stem thickness for plants with 0% and 50% leaf removal were comparable to nongrafted plants for both day 45 and 52 (Table 3-2; Figure 3-4A).

The plant height for grafted plants that underwent the 90% leaf removal method had the lowest average height and was significantly lower than the standard method on days 45 and 52 ($\alpha=0.05$; Table 3-2; Figure 3-4B). In addition, nongrafted plants were significantly taller than the 90% leaf removal grafted plants but only on day 52 and all other grafted plants were similar height to nongrafted plants on days 38, 45, and 52 ($\alpha=0.05$; Table 3-2; Figure 3-4B).

Grafted plants treated with 90% leaf removal method only displayed significant decrease in leaf area on day 38 when compared to the other 0% and 50% leaf removal methods ($\alpha=0.05$; Table 3-2; Figure 3-4C). On days 45 and 52, all grafted plants had similar leaf area whether with or without leaf removal (Table 3-2; Figure 3-4C). In addition, grafted plants also exceeded nongrafted plants in leaf area on days 38 and 45 for 50% leaf removal method, days 45 and 52 for 90% leaf removal method and all three days for the standard 0% leaf removal method ($\alpha=0.05$; Table 3-2; Figure 3-4C)

In addition to leaf area, shoot and root biomass was also reduced on day 38 for plants with 90% leaf removal grafting method compared to both 0% leaf removal grafting method as well as nongrafted plants ($\alpha=0.05$; Table 3-2; Figure 3-4D and E). On day 45, shoot biomass was similar among all treatments where as root biomass for grafted plants with 90% leaf removal was significantly less compared to the other grafted plants with 50% and 0% leaf removal ($\alpha=0.05$; Table 3-2; Figure 3-4D and E). Nongrafted plants also showed reduced root biomass compared to plants with 0% and 50% leaf removal on day 45, but on day 52 all grafted plants (0%, 50%, and 90% leaf removal) and nongrafted plants had similar root biomass (Table 3-2; Figure 3-4E). All grafted plants had similar shoot biomass on day 52 and plants that used the standard grafting method (0% leaf removal) had significantly more biomass than nongrafted plants ($\alpha=0.05$; Table 3-2; Figure 3-4D). Overall, the treatments consistently showed comparable shoot:root ratios throughout the sampling period. One exception to this trend was on day 45, when 90% leaf removal showed a higher ratio than both 0% and 50% techniques, which was mostly likely due to its low root biomass (Table 3-2).

Measurements on sampling data day 38, 45 and 52 indicated that all grafted plants (0%, 50%, and 90% leaf removal) had similar plant compactness and were the same in compactness (day 38 and 52) or more compact (day 45) than nongrafted plants ($\alpha =0.05$; Table 3-2; Figure 3-4F).

No flowers were counted on any plants until sampling day 38 post-grafting (Figure 3-5). Pollinated flowers were observed on day 45 post-grafting for plants grafted with the 0% leaf removal method as well as the nongrafted plants (data not shown). On day 52, all treatments had pollinated flowers (data not shown). Because the counts were low for the various maturity levels (buds, yellow, and pollinated), the data were combined for statistical analysis of total flower

count between treatments on days 38, 45, and 52 (Figure 3-5). All treatments were found to be similar for days 38 and 45 (Figure 3-5). On day 52, the plants that underwent 50% leaf removal had the highest mean number of flowers, which was significantly higher than the nongrafted plants, but was similar to the other grafted treatments ($\alpha = 0.05$; Figure 3-5).

Discussion

Our studies indicate that scion leaf removal can effect AR formation, transplant quality and early growth of grafted tomato transplants. Removing a large portion of the scion leaf area (90% leaf removal) during grafting consistently decreased AR formation from the scion resulting in high rates of acceptable transplant quality whether healing chamber was a high or low humidity environment. Moreover, removing a smaller proportion of scion leaves (50% leaf removal) successfully reduced AR formation and produced high rate of acceptable transplant quality but only in the shade chamber and not the plastic or humidifier chambers. Categorized AR formation rating data showed a correlating trend where the grafted plants with significant lower ratings had a higher proportion of acceptable grafts, which indicated good transplant quality. In all three healing chamber environments, the standard method of grafting (0% leaf removal) had the high ratings of AR formation as well as large proportions of unacceptable (8 % to 17%) and manageable (19% to 50%) grafted transplant quality showing great potential to increase losses of grafted transplants (Figure 3-3).

The shade chamber had the lowest RH, suggesting that humidity had an influence on the increase of AR formation on plants with 50% leaf removal in the various microclimates. Extension publications that discuss healing chamber management have attributed overly humid chambers or extended time in a humid chamber to cause AR formation on grafted scion (Johnson

et al., 2011). Our results support this claim, although our data is not replicated properly to verify it statistically. However, plants that were grafted with the 90% leaf removal method had significantly lower AR formation than 0% leaf removal in both low and high humidity chamber environments, suggesting that in addition to RH, leaf removal may influence the plant's initiation of AR following grafting. Therefore, it could be theorized that AR initiation on tomato scion is linked with the plant's endogenous reaction to stress and/or wounding and further AR growth following initiation may be exacerbated by environmental factors like humidity.

The entire process of graft union formation is fully developed in approximately 15 days (Fernández-García et al., 2004a). During the first four days post-grafting, scion and rootstock tissue at the wound site start to grow and divide to form a callus at the union; it is between days 4 and 8 when cells differentiate and reconnect vascular tissue (Fernández-García et al., 2004a). Scion tissue is especially vulnerable to water and temperature stress during this period, and has been observed to form adventitious roots during healing. Adventitious root formation occurs in plants naturally but also is promoted by several endogenous and exogenous factors, most recognizably in vegetative propagation as a response to wounding (Geiss et al., 2010; da Costa et al., 2013). The process of cutting the scion during grafting is similar to wounding in vegetative cutting production, in which propagators want to encourage rooting (da Costa et al., 2013). Maldiney et al. (1986) studies with tomato cuttings indicated that AR initials begin to appear on day 4 post-wounding, which also corresponds to the timing of vascular connection in grafted tomato.

Wounding is known to promote AR as the plant hormone auxin accumulates at the basal end of severed stems during vegetative propagation (Katsumi et al., 1969; Maldiney et al. 1986). Auxin flows basipetally and when the root system is completely removed, auxin that is produced

in the young leaves and meristematic tissue directly above the wound accumulates at the basal site (da Costa et al., 2013). Contrary to auxins, cytokinins are mostly produced in the root meristem and transported apically (Maldiney et al., 1986). A ratio of high levels of auxin and low levels of cytokinin is most notably associated with the formation of AR (da Costa et al., 2013). Results from Maldiney et al. (1986) reported that in the first three days after excising the root system from a tomato cutting, auxin levels at the base of the tomato cutting rose while cytokinin levels dramatically dropped after 24 hrs. and stayed low. It is interesting to note that the rootstock plants in our study, which underwent the same wounding process and healing chamber environment as the scion, did not form AR on hypocotyls in any of the experiments. Because of its excised shoot, the rootstock would most likely have more cytokinins accumulate at the graft wound than auxin creating low AR formation. In addition, the leaves, cotyledons, and apical meristem above the wounding site could be a source of auxin that is transported toward the graft union (Katsumi et al., 1969). Katsumi et al. (1969) showed that the removal of different proportions of cotyledon leaf surface area while leaving the apical meristem intact on cucumber cuttings reduced auxin levels at basal site and decreased AR formation. Similarly, removing leaves on young tomato seedlings prior to grafting may help reduce the amount of auxin transported to the wound site. If removing leaves decreases the amount of auxin accumulated at the graft site on the scion, it could result in less AR induction and initiation as seen in our studies, and this would further explain why AR is typically never seen on the rootstock of a grafted tomato transplant.

Although our experimental design was not replicated in the proper manner to compare chamber environment effects and/or interactions with leaf removal on AR development, we can note observed trends between the particular environments and compare them to previous studies.

Both the greenhouse facilities chamber designs utilized were identical to those utilized by Masterson et al. (in press). In the report by Masterson et al. (in press), the shade cloth chamber had significantly lower humidity and temperature than both plastic and humidifier chambers. The RH of the shade, plastic, and humidifier chambers utilized in this study were: 65%, 95%, and 95%, respectively, and similar chambers had mean values of 69%, 85%, and 91% RH, respectively (Masterson et al., in press). In addition, healing chamber studies by Johnson and Miles (2011) reported that chambers with shade cloth only had 53% RH and polyethylene film-covered chambers with and without humidifiers had 82% and 98% RH, respectively. Although our experiment was not replicated in a manner to test this question, the microclimate found within each healing chamber was similar to others that have been reported.

Leaf removal may have other effects on the transplants that prevent or promote AR formation and growth (e.g. cutting the leaves causes multiple wounding sites, potentially altering the different phytochemical response and transport). Furthermore, reducing leaf area decreases transpiration and water stress, potentially having an effect on scion tissue stress and subsequent AR formation. A complete understanding of all the particular endogenous responses to wounding and AR formation is still not clear.

Further results from studying plant growth parameters of plants grafted and healed in the shade chamber revealed that transplant quality and early plant growth can be affected by leaf removal depending on the proportion of leaf area removed and length of time post-grafting. Although, the 90% leaf removal method resulted in similar transplant height and stem diameter to other grafted plants (0% and 50% leaf removal), its reduction in leaf area and shoot biomass created less compact transplants. However, the continued growth of grafted plants with 90% leaf removal method developed significantly shorter plants with smaller stems but with similar

biomass and compactness as the grafting with no leaf removal. On the other hand, when only removing 50% of scion leaf area, grafted plants resulted in similar transplant quality as well as early growth to the standard method of 0% leaf removal except for reduced root biomass and leaf area on day 24 and 31, respectively.

One of the potential disadvantages of the leaf removal method is that removing scion leaves decreases photosynthetic leaf area, which may limit carbohydrate production during healing (Bumgarner and Kleinhenz, 2014). It is likely that reducing 90% of the leaf area during grafting in our studies also reduced the amount of carbohydrate availability in the plant, therefore, not supporting similar transplant quality and plant growth as the ones with 50% leaf removal and the plants grafted with the standard method. In addition, it may affect plant growth traits, such as stem thickness, compactness, and optimum shoot to root biomass that farmers desire in high quality transplants and early growth. Depending on the desired transplant quality attributes, propagators using the leaf removal technique to increase grafting success and reduce AR formation may consider extending transplant production time in order to strive for a higher quality transplant placed into production. Furthermore, our data suggests that it may benefit propagators using shade only chambers to keep at least 50% leaf area in order to produce higher quality grafted transplants and good early growth.

Flower counts were found similar for all leaf removal treatments compared to the standard method of grafting. Interestingly, plants grafted with the 50% leaf removal method had a higher flower count than the nongrafted plants on day 52; however, repetition of the experiment would further clarify this occurrence. These data suggest that leaf removal and grafting may have little influence on the timing of reproductive development in tomato. However, this greenhouse study occurred in a controlled environment and only included data

from the first three weeks of reproductive development and not actual fruit harvest data. Furthermore, our studies only used one scion and rootstock pairing. The same pairing of 'BHN 589' scion and 'Maxifort' rootstock has shown to increase total fruit number and scion biomass at the end of a growing season in high tunnel trials with little to no disease pressure (Masterson, 2013). Masterson (2013) also showed that removal of shoot apical meristem (i.e. complete shoot removal) of grafted tomato transplants reduced early yield when planted in high tunnels. Season extension and early production is a major focus for many growers, particularly those producing tomato fruit in high tunnels (Blomgren and Frisch, 2007; Everhart et al., 2009; Galinato and Miles, 2013; Sydorovych et al., 2013; Waterer, 2003; Wells and Loy, 1993). Therefore, a reduction in early production could decrease profitability because of the potential loss of out-of-season premium markets (Rivard and Louws, 2008; Masterson, 2013). Using a less vigorous rootstock or a different scion could produce different results for plant growth and development.

Although we used nongrafted plants in transplant quality and early growth measurements comparisons for nongrafted plants, they were more for observation and considered less valuable in evaluating the leaf removal method than comparisons with the standard grafting method (0% leaf removal). It should be noted that in this study, nongrafted plants were placed in a cool greenhouse (55 °F to 85 °F) for 10 days while the grafted plants were in the healing chamber. This protocol minimizes growth by the nongrafted plants while the grafted plants heal and allows for seeds of both treatments to be sown and subsequently transplanted on the same day. However, this process affects plant growth of the nongrafted plants in that cool nighttime temperatures keep the plants short with thick stems and compact growth as seen in our results on days evaluating for transplant quality.

Conclusions

Because grafted transplants represent a much higher investment to propagators than nongrafted plants (Lewis et al., 2014; Rivard et al., 2010b), grafting success rates and growing high quality grafts is extremely important to maximize profitability. Leaf removal during grafting can increase grafting survival rates (Masterson et al, in press) and in this study, it reduced AR development in three healing chamber environments. Any simple technique, such as leaf removal, that increases survival rates and transplant quality is highly advantageous for small-scale growers that wish to propagate their own grafted tomato plants. Furthermore, this method may better facilitate the use of healing chambers with low RH as utilized in previous studies (Johnson and Miles, 2011; Masterson et al., in press).

A clear question relates to the economics of leaf removal and how it would affect overall grafted transplant production costs with added labor for trimming. Current production cost budgets that have been reported for grafted tomato transplants estimate grafting success at 90% (Rivard et al., 2010b), which is more conservative than the grafting success rates found in these studies. Grafted plants with an unacceptable level of AR would be considered a loss and any level of AR reduces transplant quality and therefore marketability. These losses reduce profit for propagators and represent opportunity costs for tomato growers that are grafting their own plants. Propagators that graft a smaller amount of plants per year may also may find that tomato scions with trimmed leaves are easier to manage and less likely to dislodge from the grafting clip due to lower shoot weight and surface area. Research that investigates the economics of leaf removal and identifies both the costs and potential economic benefits of this technique would be valuable.

Further research is also needed to determine how auxins and other hormones play a role in AR formation on the scions of grafted plants. Retaining cotyledon leaves can contribute to AR

formation in grafted cucumber (Katsumi et al., 1969) and the location of the graft union on the scion stem and/or removal of the cotyledon leaves could further impact the development of AR on tomato scions. In addition, as more studies report the specifics of hormonal responses during graft union healing, we can gain a better understanding of when and why AR formation occurs. The results of our greenhouse study indicate that early plant growth and early flower development was not penalized by leaf removal during the grafting procedure. Additional field and/or high tunnel studies directed at investigating the effect of leaf removal during grafting on mature plant productivity would inform us more of the potential for this technique to alter early production.

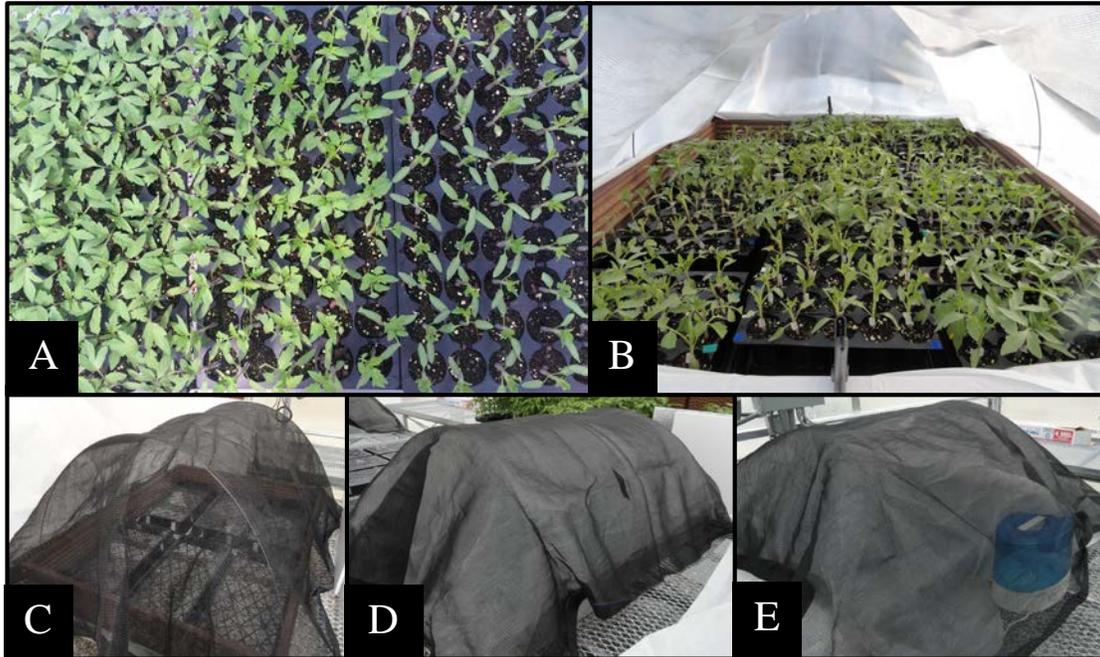


Figure 3.1 A) Leaf removal of the scion, 0%, 50%, and 90% true leaf removal (shown left to right), was applied to scion var. BHN 589 just prior to grafting to rootstock ‘Maxifort’. B) Once the plants were grafted, they were placed inside each chamber as a RCBD experiment with 20 plants per unit and four replications. Experimental repetitions were conducted in healing chambers with (C) shade cloth alone (shade), (D) shade cloth and polyethylene film (plastic), and (E) the plastic chamber with a cool-mist humidifier (humidifier).

						
0 none	1 trace root(s) ^z only	2 ≥ 1 small root(s); any trace root(s)	3 1 medium root; any small or trace root (s)	4 multiple medium roots; any small or trace root (s)	5 1 large root; any additional roots; none in soil	6 multiple large roots; any additional roots; none in soil
Acceptable				Manageable		
						
7 1 root in soil; any small or trace roots	8 1 root in soil; any medium or large roots	9 multiple roots in soil; any additional roots	10 multiple roots in soil; scion growth promoted by adventitious roots; rootstock not functioning			
Unacceptable						

Figure 3-2: A rating scale 0-10 was used to describe the degree of adventitious root formation from the scion and determine the marketable quality of the transplant. Pictures were taken on day 24 post-grafting.

^ztrace root < 2 mm ≤ small root < 5 mm ≤ medium root ≤ 10 mm ≤ large root

Table 3-1: Determined adventitious root ratings using a scale from 0 to 10 based on size and length of roots during days post-grafting and resulting fresh weight of adventitious roots.

Treatment ^z	Average AR Rating (per plant) ^{yv}			Fresh Weight of AR (mg/plant) ^w
	Day 10 ^w	Day 17	Day 24	
<i>Shade Chamber^x</i>				
0% LR	1.62 a ^u	2.21 a	2.49 a	38.13 a
50% LR	0.31 b	0.45 b	0.55 b	0.76 b
90% LR	0.05 b	0.12 b	0.16 b	0.00 b
<i>Plastic Chamber</i>				
0% LR	2.99 a	3.47 a	3.52 a	49.07 a
50% LR	2.18 a	2.51 a	2.60 a	17.33 a
90% LR	0.59 b	0.75 b	0.81 b	1.63 b
<i>Humidifier Chamber</i>				
0% LR	4.10 a	4.11 a	4.19 a	39.79 a
50% LR	2.73 ab	2.94 a	3.03 a	22.41 a
90% LR	0.91 b	1.04 b	1.10 b	1.00 b

^zTreatments include percentage of leaf removal (LR) removed from the scion tomato var. BHN 589 during grafting onto rootstock var. Maxifort

^yExperimental design including RCBD with 20 plants in each experimental unit.

^xExperiment was performed in 3 different chambers described in fig. 3-1

^wData was collected on days 10, 17 and 24 post-grafting. On day 24, adventitious roots (AR) were excised and weighed

^vAdventitious root (AR) ratings were determined using a scale from 0 to 10 (shown in fig. 3-2) based on size and length of roots on days 10, 17, and 24 post-grafting

^uDifferent letters represent significance based on Tukey's $\alpha = 0.05$. Data was analyzed independently for chamber and day.

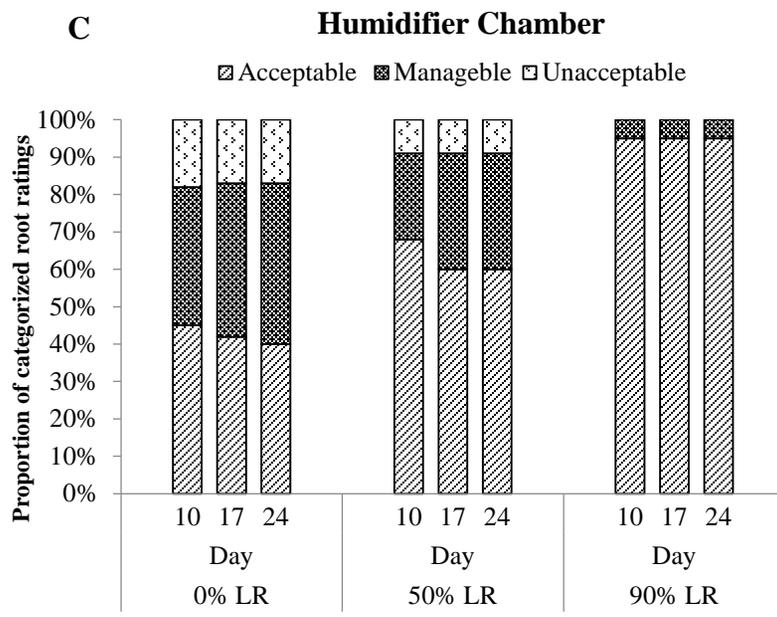
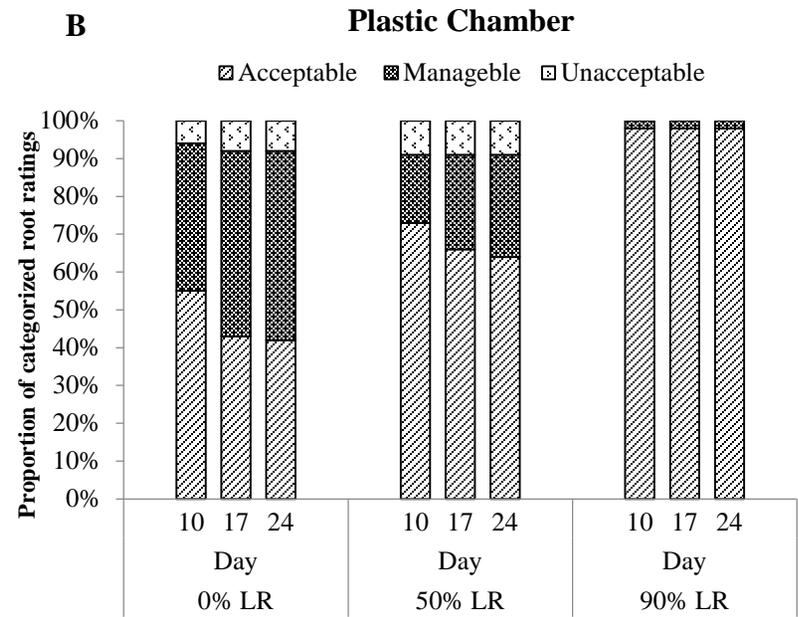
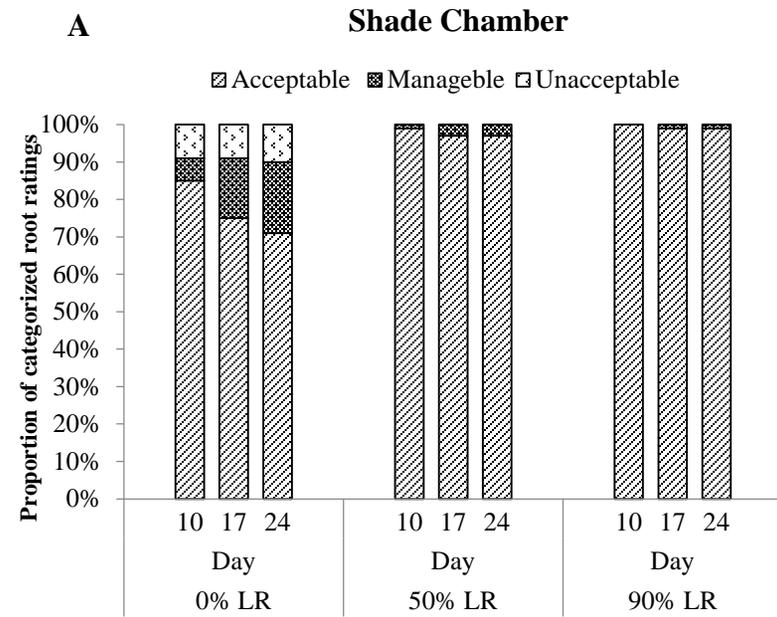


Figure 3-3: Proportions of the quality of grafted tomato transplants determined by AR formation on days 10, 17, and 24 days post-grafting in (A) shade, (B) plastic, and (C) humidifier chamber. Using the scale in Figure 3-2, ratings of 0-3 were categorized as acceptable quality; ratings of 4-6 were manageable quality; and ratings of 7-10 were unacceptable quality.

Table 3-2: Growth measurements of nongrafted tomato and grafted tomato plants with varying levels of leaf removal grown in a greenhouse at the Olathe Horticulture Research and Extension Center

Treatment	Stem (mm) ^v		Height (cm)		Leaf Area (cm ²)		Shoot Biomass (g)		Root Biomass (g)		Shoot:Root Ratio		Compactness (mg/cm)	
<i>Day 24^w</i>														
0% LR ^{z,y,x}	4.5	ab ^u	15.4	ab	110	a	0.84	b	0.17	ab	5.02	ab	55.1	ab
50% LR	4.2	b	13.8	ab	91	ab	0.67	bc	0.11	c	6.98	a	48.4	bc
90% LR	4.0	b	11.7	b	77	b	0.48	c	0.13	bc	3.65	b	41.2	c
Nongrafted	5.1	a	16.9	a	117	a	1.11	a	0.19	a	5.80	ab	65.2	a
<i>Day 31</i>														
0% LR	4.9	b	18.2	ab	188	a	1.20	b	0.46	ab	2.62		65.9	b
50% LR	4.9	b	16.4	ab	170	b	1.07	b	0.40	b	2.75		65.3	b
90% LR	4.4	b	15.1	b	149	c	0.86	c	0.31	c	2.76		57.0	c
Nongrafted	5.7	a	19.0	a	174	ab	1.45	a	0.50	a	2.89		76.2	a
<i>Day 38</i>														
0% LR	6.4	ab	22.5		343	a	2.76	a	0.87	a	3.21		123.4	
50% LR	6.1	bc	21.0		313	a	2.55	ab	0.76	ab	3.35		121.6	
90% LR	5.6	c	19.5		260	b	2.11	b	0.61	b	3.45		108.5	
Nongrafted	7.1	a	23.1		267	b	2.66	a	0.87	a	3.13		115.2	
<i>Day 45</i>														
0% LR	8.8	a	41.9	a	1850	a	10.35		2.88	a	3.61	b	246.8	a
50% LR	8.2	ab	39.0	ab	1873	a	9.97		2.74	a	3.64	b	256.3	a
90% LR	8.1	b	36.5	b	1676	a	9.18		2.02	b	4.57	a	251.7	a
Nongrafted	8.9	a	39.4	ab	1335	b	8.21		1.95	b	4.22	ab	208.6	b
<i>Day 52</i>														
0% LR	10.3	a	58.8	a	3387	a	37.30	a	7.39		5.10		633.6	
50% LR	9.9	ab	57.0	ab	3118	ab	35.15	ab	6.79		5.26		616.9	
90% LR	9.5	b	54.3	b	3216	a	33.65	ab	6.39		5.41		619.9	
Nongrafted	10.4	a	58.6	a	2696	b	30.45	b	5.78		5.27		519.7	

^xTreatments include ‘BHN 589’ scion plants with 0%, 50%, and 90% leaf removal (LR) grafted onto rootstock ‘Maxifort’ and ‘BHN 589’ nongrafted plants.

^yScion and nongrafted plants were planted on 19 March, two days before rootstock plants. All plants were grown under typical greenhouse optimal growing conditions for tomato (64 °F to 82 °F) except during days 1 to 10 post-grafting.

^zFor days 1 to 10 post-grafting (using splice-grafting method), grafted plants were placed in shade cloth only healing chamber (avg. 68% RH and 68 °F) while nongrafted plants were placed in a non-shaded but cooler environment (55 °F to 85 °F).

^wExperiment was arranged in a RCBD with 4 blocks and 15 plants in each experimental unit. Destructive sampling of 3 plants per unit occurred on days 24, 31, 38, 45, and 51 post-grafting.

^vData was analyzed using a mix model with selected covariance structure based on residuals for each growth parameter: stem and height data used first-order autoregressive; leaf area, root biomass, and compactness data used heterogeneous compound symmetry and shoot biomass and shoot-to-root ratio used heterogeneous first-order autoregressive.

^uDifferent letters show significant differences between values when using the Bonferroni method to compare LSDs independently of day with $\alpha=0.05$.

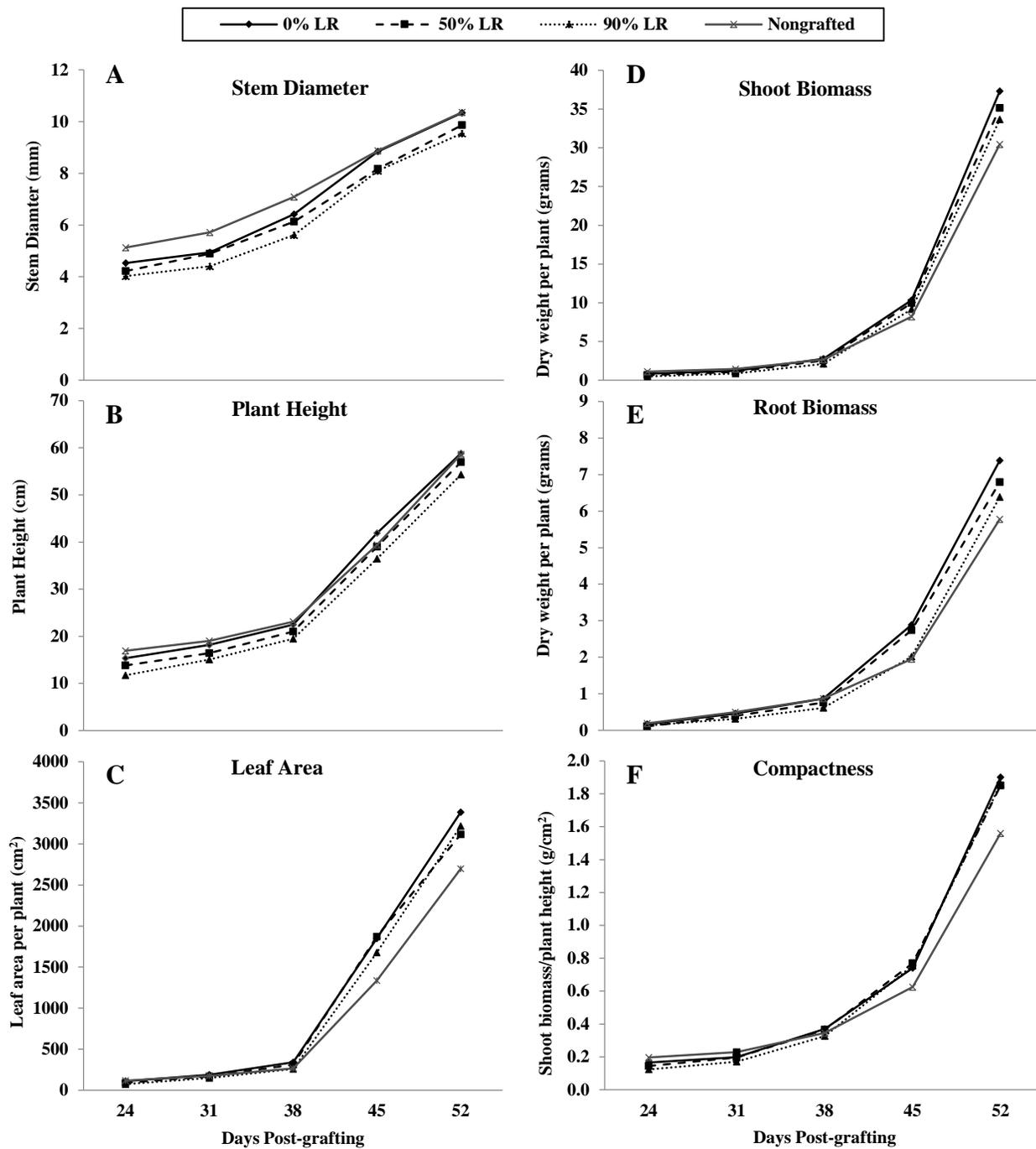


Figure 3-4: Nongrafted ‘BHN 589’ tomato plants and grafted plants with ‘BHN 589’ scion and ‘Maxifort’ rootstock were treated with 0%, 50% or 90% leaf removal of scion leaf tissue at the time of grafting and placed in a shade cloth only healing chamber (avg. 68% RH and 68 °F) for 10 days and then arranged as a RCBD experiment that was conducted in a greenhouse at the Olathe Horticulture Research and Extension Center. On days 24, 31, 38, 45 and 52 post-grafting, 3 randomly sampled plants for each treatment for each of the 4 blocks were destructively measured for the following growth parameters: (A) stem diameter; (B) plant height; (C) leaf area; (D) shoot biomass; (E) root biomass; and (F) compactness.

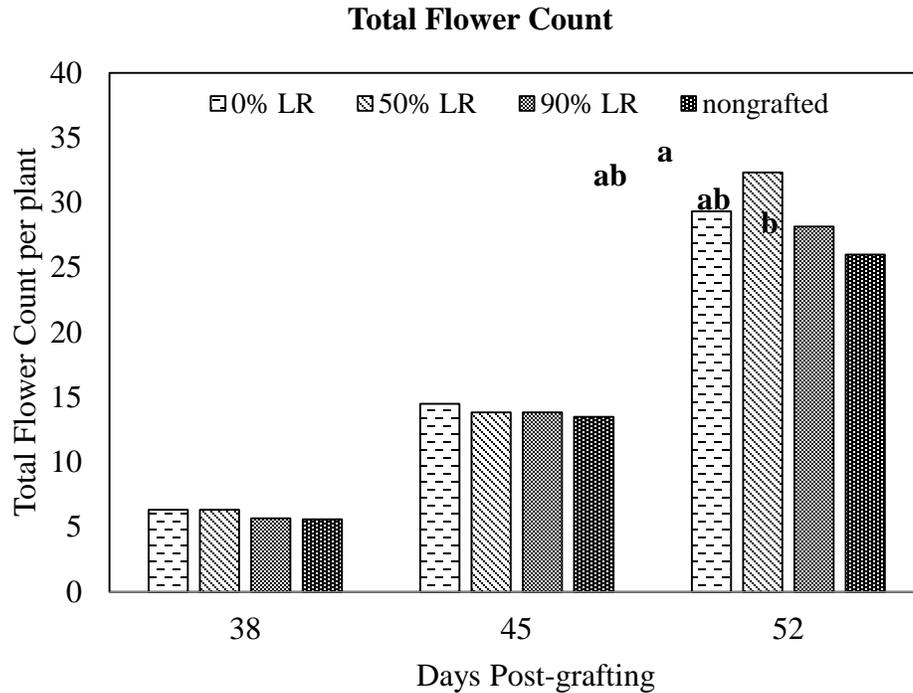


Figure 3-5 Total flower count of plants grafted with 0%, 50%, and 90% leaf removal (LR) as well as nongrafted plants when seeds are planted on the same day. Flower count was determined using 3 randomly sampled plants for each treatment for each of the 4 blocks in a RCBD experiment that was conducted in a greenhouse at the Olathe Horticulture Research and Extension Center. Flower count data, y , was transformed where $y^* = \sqrt{y + \frac{3}{8}}$, and analyzed independently for days 38, 45, and 52 using a mix model with the covariance structure heterogeneous compound symmetry and Poisson distribution. Different letters indicate significant differences with comparisons using the Bonferroni method when $\alpha=0.05$.

Bibliography

- Barrett, C.E., X. Zhao, and A. Hodges. 2012a. Cost benefit analysis of using grafted transplants for root-knot-nematode management in organic heirloom tomato production. *HortTechnology* 22(2):252-257.
- Barrett, C.E., X. Zhao, C.A. Sims, J.K. Brecht, E.Q. Dryer, and Z. Gao. 2012b. Fruit composition and sensory attributes of organic heirloom tomatoes as affected by grafting. *HortTechnology* 22(6):804-809.
- Barrett, C.E., X. Zhao, and R. McSorley. 2012c. Grafting for root-knot nematode control and yield improvement in organic heirloom tomato production. *HortScience* 47(5):614-620.
- Bausher, M. 2011. Grafting technique to eliminate rootstock sucking of grafted tomato. *HortScience* 46(4):596-598.
- Bausher, M. 2013. Graft angle and its relationship to tomato plant survival. *HortScience* 48(1):34-36.
- Besri, M. 2003. Tomato grafting as an alternative to methyl bromide in Morocco. Institut Agronomique et Vétérinaire Hassan II, Morocco. 23 Feb. 2016 < <https://mbao.org/static/docs/confs/2003-sandiego/papers/012%20besrimgraftingmbao2003sd.pdf>>
- Blomgren, T. and T. Frisch. 2007. High tunnels: Using low-cost technology to increase yields, improve quality, and extend the season. University of Vermont Center of Sustainable Agriculture. 26 Aug. 2015. <<https://www.uvm.edu/~susagctr/resources/HighTunnels.pdf>>
- Both, A.J., E. Reiss, J.F. Sudal, K.E. Holmstrom, C.A. Wyenandt, W.L. Kline, and S.A. Garrison. 2007. Evaluation of a manual energy curtain for tomato production in high tunnels. *HortTechnology* 17(4):467-472.
- Buller, S., D. Inglis, and C. Miles. 2013. Plant growth, fruit yield and quality, and tolerance to verticillium wilt of grafted watermelon and tomato in field production in the pacific northwest. *HortScience* 48(8):1003-1009.
- Bumgarner, N.R. and M.D. Kleinhenz. 2014. Grafting guide – A pictorial guide to the cleft and splice graft methods. The Ohio State Univ. Ohio Agr. Res. Dev. Ctr. Bul 950.
- Carey, E.E., L. Jett, W.J. Lamont Jr., T.T. Nennich, M.D. Orzolek, and K.A. Williams. 2009. Horticultural crop production in high tunnels in the United States: A Snapshot. *HortTechnology* 19(1):37-43.
- Colla, G., Y. Rouphael, C. Leonardi, and Z. Bie. 2010. Role of grafting in vegetable crops grown under saline conditions. *Sci. Hortic.* 127(2):147-155.
- Conner, D. S., K. B. Waldman, A. D. Montri, M. W. Hamm, and J. A. Biernbaum. 2010. Hoophouse contributions to economic viability: Nine Michigan case studies. *HortTechnology* 20(5):877-884.
- Davis, A.R., P. Perkins-Veazie, R. Hassell, A. Levi, S.R. King, and X. Zhang. 2008. Grafting effects on vegetable quality. *HortScience* 43(6):1670-1672.
- da Costa, C.T., M.R. de Almeida, C.M. Ruedell, J.Schwambach, F.S. Marachin, and A.G. Fett-Neto. 2013. When stress and development go hand in hand: main hormonal controls of adventitious rooting in cuttings. *Frontiers in Plant Science* 4(133):1-19.
- de Klerk, G., W. van der Krieken, and J.C. de Jong. 1999. Review: The formation of adventitious roots: New concepts, new possibilities. *In Vitro Cellular and Developmental Biology Plant* 35(3):189-199.
- Djidonou, D., Z. Zhao, E.H. Simonne, K.E. Koch, and J.E. Erickson. 2013. Yield, water-, and nitrogen-use efficiency in field-grown, grafted tomatoes. *HortScience* 48(4):485-492.
- Eigenbrod, C. and N. Gruda. 2015. Urban vegetable for food securities in cities: A review. *Agron. Sustain. Dev.* 35:483-498.
- Everhart, E., R. Hansen, D. Lewis, L. and Naeve, H. Taber. 2009. Iowa high tunnel fruit and vegetable production manual. Leopold Center for Sustainable Agriculture, Iowa State Univ. Sci. and Technol. 31 Jan. 2016 <

<https://www.leopold.iastate.edu/sites/default/files/pubs-and-papers/2010-01-iowa-high-tunnel-fruit-and-vegetable-production-manual.pdf>>

- Eklöf, S., C. Åstot, J. Blackwell, T. Moritz, O. Olsson, and G. Sandberg. 1997. Auxin-cytokinin interactions in wild-type and transgenic tobacco. *Plant Cell Physiol.* 38(3):225-235.
- Fernández-García, N., G. M. Carvajal, and E. Olmos. 2004a. Graft union in tomato plants: Peroxidase and catalase involvement. *Annals of Botany* 93: 53-60.
- Fernández-García, N., V. Martínez, A.Cerdá, and M. Carvajal. 2004b. Fruit quality of grafted tomato plants grown under saline conditions. *J. of Hort. Sci. and Biotechnol.* 79(6):995-1001.
- Flores, F.B., P. Sanchez-Bel, M.T. Estañ, M.M. Martinez-Rodriguez, E. Moyano, B. Morales, J.F. Campos, J.O. Garcia-Abellán, M.I. Egea, N. Fernández-García, F. Romojaro, and M.C. Bolarín. 2010. The effectiveness of grafting to improve tomato fruit quality. *Scientia Horticulturae* 125:211-217.
- Foley, J.A., N. Ramankutty, K.A. Brauman, E.S Cassidy, J.S. Gerber, M. Johnson, N.D. Mueller, C. O'Connell, D.K. Ray, P.C. West, C. Balzer, E.M. Bennett, S.R. Carpenter, J. Hill, C. Mondfreda, S. Polasky, J. Rockström, J. Sheeham, S. Siebert, D. Tilman, and D.P. Zaks. 2011. Solutions for a cultivated planet. *Nature* 478: 337-342
- Galinato, S.P. and C.A. Miles. 2013. Economic profitability of growing lettuce and tomato in western Washington under high tunnel and open-field production systems. *HortTechnology* 23(4):453-461.
- Geiss G., L. Gutierrez and C. Bellini. 2010. Adventitious root formation: new insights and perspectives, p127-156. In: T. Beekman (ed.) *Root development*. Wiley-Blackwell, Ames, IA.
- Gilardi, G. M.L. Gullion, and A. Garibaldi. 2013. Critical aspects of grafting as a possible strategy to manage soil-borne pathogens. *Scientia Horticulturae* 149:19-21.
- Gilardi, G., P. Colla, M. Pugliese, M. Baudino, M.L. Gullino and A. Garibaldi. 2014. Control of *Colletotrichum coccodes* on tomato by grafting and soil amendments. *J. Phytopathol.* 162:116-123.
- Guan, W., X. Zhao, R. Hassell, and J. Thies. 2012. Defense mechanisms involved in disease resistance of grafted vegetables. *HortScience* 47(2):164-170.
- Hanson, B.R., S.T. Grattan, and A. Fulton. 2006. Agricultural salinity and drainage. Univ. California Irrigation Program. 27 Aug. 2015 < http://hos.ufl.edu/sites/default/files/faculty/gdliu/HansonGrattan2006_0.pdf>.
- He, Y., Z. Zhu, J. Yang, X. Ni, and B. Zhu. 2009. Grafting increases the salt tolerance of tomato by improvement of photosynthesis and enhancement of antioxidant enzymes activity. *Environ. and Expt. Bot.* 66:270-278.
- Hodgson, K., M.C. Campbell, and M. Bailkey. 2011. *Urban agriculture: Growing healthy, sustainable places*. American Planning Assoc., Chicago, IL.
- Hunt, A.R. 2006. Consumer interactions and influences on farmers' market vendors. *Renewable Agr. and Food Systems*: 22(1): 54-66.
- Hunter, B., D. Drost, B. Black, and R. Ward. 2012. Improving growth and productivity of early-season high-tunnel tomatoes with targeted temperature additions. *HortScience* 47(6):733-740.
- Johnson, S.J. and C.A. Miles. 2011. Effect of healing chamber design on the survival of grafted eggplant, tomato, and watermelon. *HortTechnology* 21(6):752-758.
- Johnson, S.J., P. Kreider, and C.A. Miles. 2011. Vegetable grafting: the healing chamber. Wash. St. Univ. Ext. Factsheet FS051E.
- Jordan, J. 2007. The heirloom tomato as cultural object: Investigating taste and space. *Sociol. Ruralis* 47:20-41.
- Katsumi, M., Y. Chiba and M. Fukuyama. 1969. The roles of the cotyledons and auxin in the adventitious root formation of hypocotyl cuttings of light-grown cucumber seedlings. *Physiol. Plantarum* 22:993-1000.

- Kevers, C., J. Hausman, O. Faivre-Rampant, D. Evers, and T. Gaspar. 1997. Hormonal control of adventitious rooting: Progress and questions. *J. Appl. Bot. -Angew. Bot.* 71(3-4):71-79.
- King, S., A. Davis, W. Liu, and A. Levi. 2008. Grafting for disease resistance. *HortScience* 43(6):1673-1676
- Knewton, S.J.B., E.E. Carey, and M.B. Kirkham. 2010a. Management practices of growers using high tunnels in the Central Great Plains of the United States. *HortTechnology* 20(3):639-645.
- Knewton, S.J.B., R. Janke, M.B. Kirkham, K.A. Williams, and E.E. Carey. 2010b. Trends in soil quality under high tunnels. *HortScience* 45(10):1534-1538.
- Knewton, S.J.B., M.B. Kirkham, R.R. Janke, L.W. Murray, and E.E. Carey. 2012. Soil quality after eight years under high tunnels. *HortScience* 47(11):1630-1633.
- Kubota, C., M.A. McClure, N. Kokalis-Burelle, M.G. Bausher, and E.N. Roskopf. 2008. Vegetable grafting: History, use, and current technology status in North America. *HortScience* 43(6):1664-1669.
- Leonardi C., F. Giuffrida. 2006. Variation of plant growth and macronutrient uptake in grafted tomatoes and eggplants on three different rootstocks. *Europ. J. Hort. Sci.* 71(3):97-101.
- Lamont, W. 2009. Overview of the use of high tunnels worldwide. *HortTechnology* 19(1):25-29.
- Lamont, W.J., Jr. 2013. High tunnel construction and production in a large metropolitan city. *Acta Hort.* 987:45-47.
- Lee, J. 1994. Cultivation of grafted vegetables. I. Current status, grafting methods, and benefits. *HortScience* 29(4):235-239.
- Lee, J., H. Bang, and H. Ham. 1998. Grafting of vegetables. *J. Jpn. Soc. Hortic. Sci.* 67(6):1098-1104.
- Lee, J., C. Kubota, S.J. Tsao, Z. Bie, P. Hoyos Echevarria, L. Morra, and M. Oda. 2010. Current status of vegetable grafting: Diffusion, grafting techniques, automation. *Sci. Hort.* 127(2):93-105.
- Lewis, M., C. Kubota, R. Tronstand, and Y. Son. 2014. Scenario-based cost analysis for vegetable grafting nurseries of different technologies and sizes. *HortScience* 49(7):917-930.
- Louws, F.J., C.L. Rivard, and C. Kubota. 2010. Grafting fruiting vegetables to manage soilborne pathogens, foliar pathogens, arthropods and weeds. *Scientia Hort.* 127(2):127-146.
- Maldiney, F., F.G. Sotta, L. Sossountzov and E. Miginiac. 1986. Endogenous levels of abscisic acid, indole-3-acetic acid, zeatina and zeatin-riboside during the course of adventitious root formation in cuttings of *Craigella* and *Craigella* lateral suppressor tomatoes. 1986. *Physiol. Plantarum* 68: 426-430.
- Martínez-Ballesta, M.C., C Alcaraz-López, B. Muries, C. Mota-Cadenas, and M. Carvajal. 2010. Physiological aspects of rootstock-scion interactions. *Scientia Hort.* 127:112-118.
- Martellozzo, F., J. Landry, D. Plouffe, V. Seufert, P. Rowhani and N. Ramankutty. 2014. Urban agriculture: a global analysis of the space constraint to meet urban vegetable demand. *Environ. Res. Lett.* 9. 7 Feb. 2016. <http://iopscience.iop.org/article/10.1088/1748-9326/9/6/064025/pdf>.
- Masterson, S. 2013. Propagation and utilization of grafted tomatoes in the Great Plains. Kan. St. Univ., Manhattan, M.S. Thesis. < <https://krex.k-state.edu/dspace/handle/2097/16912>>.
- Masterson, S.A., M.M. Kennelly, R.R. Janke, and C.L. Rivard. In press. The impact of scion shoot removal and rootstock cultivar on early yield and vigor of grafted tomatoes in high tunnels in the Great Plains.
- McAvoy, T., J.H. Freeman, S.L. Rideout, S.M. Olson, and M.L. Paret. 2012. Evaluation of grafting using hybrid rootstocks for management of bacterial wilt in field tomato production. *HortScience* 47(5):621-625.
- Montri, A. and J.A. Biernbaum. 2009. Management of the soil environment in high tunnels. *HortTechnology* 19(1):34-36.
- Nobuoka, T., T. Nishimoto and K. Toi. 2005. Wind and light promote graft-take and growth of grafted tomato seedlings. *J. Japan Soc. Hort. Sci.* 74(2):170 (abstr.)

- Nordström, A. and L. Eliasson. 1991. Levels of endogenous indole-3-acetic acid and indole-3-acetylaspatic acid during adventitious root formation in pea cuttings. *Physiol. Plantarum* 82:599-605.
- O'Connell, S., C. Rivard, M.M. Peet, C. Harlow, and F. Louws. 2012. High tunnel and field production of organic heirloom tomatoes: Yield, fruit quality, disease, and microclimate. *HortScience* 47(9):1283-1290.
- Oda, M. 1999. Grafting of vegetables to improve greenhouse production. *Food & Fert. Technol. Ctr. Ext. Bul.* 480:1-11.
- Oda, M. 2002. Grafting of vegetable crops. *Sci. Rep. Agr. and Biol. Sci. Osaka Pref. Univ.* 54:49-72.
- Rouphael, Y., D. Schwarz, A. Krumbein, and G. Colla. 2010. Impact of grafting on product quality of fruit vegetables. *Scientia Hort.* 127:172-179.
- Rivard, C.L and F.J. Louws. 2006. Tomato grafting for disease resistance in heirloom tomatoes. *NC St. Univ. and NC Coop. Ext. Serv. Bul.* E07 45829.
- Rivard, C.L. and F.J. Louws. 2008. Grafting to manage soilborne diseases in heirloom tomato production. *HortScience* 43(7):2104-2111.
- Rivard, C.L and F.J. Louws. 2011. Tomato grafting for disease resistance and increased productivity. *SARE. Factsheet GS05-046.*
- Rivard, C.L., S. O'Connell, M.M. Peet, R.M. Welker, and F.J. Louws. 2012. Grafting tomato to manage bacterial wilt caused by *Ralstonia solanacearum* in the southeastern United States. *Plant Dis.* 96(7):973-978.
- Rivard, C.L., S. O'Connell, M.M. Peet, and F.J. Louws. 2010a. Grafting tomato with interspecific rootstock to manage diseases caused by *Sclerotium rolfsii* and southern root-knot nematode. 2010a. *Plant Dis* 94(8):1015-1021.
- Rivard, C.L., O. Sydorovych, S. O'Connell, M.M. Peet, and F.J. Louws. 2010b. An economic analysis of two grafted tomato transplant production systems in the United States. *HortTechnology* 20(4):794-803.
- Rivero, R.M., J.M. Ruiz, and L. Romero. 2003a. Can grafting in tomato plants strengthen resistance to thermal stress? *J. Sci. Food Agric.* 83(13):1315-1319.
- Rivero, R.M., J.M. Ruiz and L. Romero. 2003b. Role of grafting in horticultural plants under stress conditions. *Food, Agr. & Environ.* 1(1):70-74.
- Rogers, M.A. and A.L. Wszelaki. 2012. Influence of high tunnel production and planting date on yield, growth, and early blight development on organically grown heirloom and hybrid tomato. *HortTechnology* 22(4):452-462.
- Rogus, S. and C. Dimitri. 2014. Agriculture in urban and peri-urban areas in the United States: Highlights from the Census of Agriculture. *Renewable Agr. and Food Systems* 30(1):64-78.
- Rysin, O., C. Rivard, and F.J. Louws. 2015. Is vegetable grafting economically viable in the United States: Evidence from four different tomato production systems. *Acta Hort.* 1086: 79-86.
- Sánchez, E. 2015. Dealing with high soluble salt levels in high tunnels. *Penn St. Ext. Factsheet* 25 Feb. 2016. <<http://extension.psu.edu/plants/vegetable-fruit/fact-sheets/salts-in-high-tunnels/dealing-with-high-soluble-salt-levels-in-high-tunnels>>.
- Savvas, D., A. Savva, G. Ntatsi, A. Ropokis, I. Karapanos, A. Krumbein, and C. Olympios. 2011. Effects of three commercial rootstocks on mineral nutrition, fruit yield, and quality of salinized tomato. *J. Plant Nutr. Soil Sci.* 174:154-162.
- Savvas, D., D. Papastavrou, G. Ntatsi, A. Ropokis, C. Olympios, H. Hartmann, and D. Schwarz. 2009. Interactive effects of grafting and manganese supply on growth, yield, and nutrient uptake on tomato. *HortScience* 44(7):1978-1982.
- Savvas, D., G. Colla, Y. Rouphael, and D. Schwarz. 2010. Amelioration of heavy metal and nutrient stress in fruit vegetables by grafting. *Scientia Hort.* 127:156-161.
- Schwarz, D., G.B. Öztekin, Y. Tüzel, B. Brückner, and A. Krumbein. 2013. Rootstocks can enhance tomato growth and quality characteristics at low potassium supply. *Scientia Hort.* 149:70-79.

- Schwarz, D., Y. Roupael, G. Colla, and J.H. Venema. 2010. Grafting as a tool to improve tolerance of vegetables to abiotic stresses: Thermal stress, water stress and organic pollutants. *Scientia Hort.* 127(2):162-171.
- Smit, J., J. Nasr, and A. Ratta. 2001. Urban agriculture: Food, jobs, and sustainable cities. The Urban Agriculture Network. Available online <http://www.jacsmit.com/book.html>
- Sydorovych, O., C.L. Rivard, S. O'Connell, C.D. Harlow, M.M. Peet, and F.J. Louws. 2013. Growing organic heirloom tomatoes in the field and high tunnels in North Carolina: Comparative economic analysis. *HortTechnology* 23(2):227-236.
- Taiz, L. and E. Zeiger. 2002. *Plant physiology*. 3rd ed. Sinauer, Sunderland, MA.
- United Nations. 2014. World urbanization prospects: The 2014 revision highlights. Dept. Economic and Social Affairs. 29 Oct. 2015, < <http://esa.un.org/unpd/wup/Publications/Files/WUP2014-Highlights.pdf>>
- U.S. Dept. Agr. 2014. Cleveland high tunnel initiative. Fact sheet. NRCS. 25 Feb. 2016
http://www.nrcs.usda.gov/wps/portal/nrcs/detail/oh/programs/?cid=nrcs144p2_029508
- U.S. Dept. Agri. (n.d.) NRCS high tunnel initiative. 20 Apr. 2016.
<<http://www.nrcs.usda.gov/wps/portal/nrcs/detail/national/programs/financial/equip/?&cid=stelprdb1046250>>.
- U.S. Dept. Health and Human Serv. and U.S. Dept. Agri.. 2015 – 2020 Dietary Guidelines for Americans. 8th Ed. 25 Feb. 2016.
<<http://health.gov/dietaryguidelines/2015/guidelines/>>.
- Vu, N., C Zhang, Z. Xu, Y. Kim, H. Kang, and I. Kim. 2013. Enhanced graft-take ratio and quality of grafted seedlings by controlling temperature and humidity conditions. *Protected Hort. and Plant Factory* 22 (2): 146-153.
- Waterer, D. 2003. Yields and economics of high tunnels for production of warm-season vegetable crops. *HortTechnology* 13(2): 339-343.
- Wells, O.S. and J.B. Loy. 1993. Rowcovers and high tunnels enhance crop production in the northeastern United States. *HortTechnology* 3(1):92-95.
- Wittwer, S.H. 1993. World-wide use of plastics in horticultural production. *HortTechnology* 3(1):6-19.
- Zhao, X., E.E. Carey, J.E. Young, W.W. Wang, and T. Iwamoto. 2007. Influences of organic fertilization, high tunnel environment, and postharvest storage on phenolic compounds in lettuce. *HortScience* 42(1):71-76