Improved Tomato Grafting Technologies

DISSERTATION

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By

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Abstract

Seedling development is a period of dynamic change within the overall vegetable plant and crop development period. For example, leaf area, stem length, and above-ground biomass can increase several fold within 1-3 weeks of emergence, possibly signaling high rates of carbon fixation on a per gram fresh weight basis and prescribed patterns of primary growth. Still, while general patterns of seedling development are familiar and the influence of individual major environmental factors on it are well chronicled, a reliable, accessible, and highly repeatable approach to describe the efficiency with which seedlings convert growth factors into biomass and partition it is unavailable. We hypothesized that plant and environmental data could be integrated into a single "seedling vigor" value allowing for more direct and consistent comparisons of seedling growth within and across experiments. We tested this hypothesis in a greenhouse experiment involving the simultaneous tracking of seven parameters of seedling growth (above-ground biomass and growth pattern) in twenty-three commercial tomato varieties and four environmental variables through 18 d after seeding. A formula created for the test used the plant and environmental data in calculating seedling vigor values and the experiment was repeated twice over a four-month period in the spring. Minimum and maximum seedling vigor values differed 79- to 575-fold among cultivars in runs 1 and 2, respectively, although relative variety vigor values were generally consistent between runs. These results demonstrate: 1) that varieties

differ in their primary growth capacities under identical growing conditions and 2) that calculations of vigor like the one demonstrated here can reliably differentiate these capacities and help standardize reports including them.

Normal seedling development, perhaps especially root-shoot communication and root and shoot level (carbon) partitioning, is severely disrupted in the process of grafting. In fact, grafting requires two steps that are very likely to alter normal seedling developmental patterns and, possibly, subsequent vegetative and reproductive growth. The first stage of grafting is to prepare seedlings of at least two varieties to exacting specifications, a process that can be made more efficient and reliable by a greater understanding of factors influencing vigor, as described earlier. Later stages involve cutting and connecting portions of the two seedlings then facilitating the single plant's healing as a newly-made physical hybrid. Employing whole ungrafted seedlings as controls, experiments exposing newly-grafted plants to various temperature and light conditions during healing offer two benefits. First, they strengthen understanding of abiotic influences on whole seedling growth through roughly 40 days after seeding (an under-studied period). Second, comparing control and grafted-plant values, these experiments help determine when and to what extent grafted plants reach normal developmental milestones and vascular capacity. The latter outcome requires an appropriate protocol for assessing seedling/grafted plant vascular capacity, a focus of a portion of this program.

Vegetable seedlings are fully or partially mechanically defoliated immediately before grafting and their stems are severed before being 'splinted' to provide stability during healing. Collectively, the process: a) nullifies investments seedlings made in leaf and root production before grafting; b) disrupts water relations, growth regulator balances, and other aspects of physiology; c) requires more or less immediate reestablishment of fully functional vascular connections between root and shoot, a prerequisite for further vegetative growth and, later,

fruiting under potentially stressful conditions; and d) delays the readiness of grafted plants for use in commercial production relative to standard ungrafted plants, also causing grafted plants to be more costly to produce than ungrafted ones. Overall, newly-grafted plants are expected to require photosynthetically-derived energy to heal and resume growth. However, it is also reasonable to expect them to have a diminished photosynthetic capacity and, possibly, tolerance to even moderate light and temperature levels. Regardless, it is important to document light and temperature effects on healing and regrowth, especially using approaches uncommon in much of the horticultural literature.

Therefore, a quantitative method to monitor healing was developed then used to test the effects of pre- and post-grafting light levels on healing. Dye movement used to visualize trans-graft union, root to shoot water movement and stem elongation were used to track plant condition. Both were greater in newly-grafted plants exposed to extended periods of light versus dark soon after grafting. Follow-up studies including a total of ten temperature-light intensity combinations as treatments during the healing period were completed. Plants exposed to moderate to high light levels (i.e., 150-300 $\mu mol/m^2/s$) of light and moderate temperatures achieved the largest aboveground dry weight and compactness. These results suggest that, in practice, the benefits of promoting photosynthesis beginning immediately after grafting exceed the benefits of protecting against potentially damaging effects of light exposure, provided moderate temperatures can be maintained. The data also call for follow-up experiments regarding, for example, the effects of wavelength, duration, and other components of irradiance on core aspects of healing and short-term regrowth.

Interest in grafted plants stems partly from the expectation that their root systems are better able to acquire nutrients and water, resist abiotic and biotic stresses, and display more vigor that the root systems of the scion cultivar. If true, the optimal cultural, nutrient, and irrigation

management plans of grafted and ungrafted plants and their responses to individual combinations of growing factors are likely to differ. In another experiment, grafted plants representing three rootstock-scion combinations (3 rootstock, 1 scion) and ungrafted plants of the same scion variety were grown in open field, conventionally-managed plots containing one of two fertilization treatments (pre-plant fertilization only, pre-plant fertilization with standard fertigation). The fertilization treatment-grafting interaction was not significant. Yield tended to be higher in plots containing grafted versus ungrafted plants, regardless of fertilization treatment. ˚Brix was higher in fruits from ungrafted versus grafted plants, while trends in pH and titratable acidity were inconsistent. These results support the emerging hypothesis that grafted plants are more productive than ungrafted ones under a range of soil conditions but that fruit chemical characteristics may also be altered by grafting.

Dedicate to my beloved family

Especially to the memory of my dearest father

& to my loving and supportive husband

You are the reasons for what I have done right

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Fields of Study

Major Field: Horticulture and Crop Science

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Chapter 1: Introduction

Grafting has been used in agriculture since ancient times, with fruit trees being a typical example of crops commonly grafted in early agricultural societies (Webster, 1995). Grafting allows growers to immediately achieve the benefits of two plants, one providing a root system (rootstock) and the other providing a shoot (scion). While well-established for fruits and trees, this technique was first introduced into vegetable production when watermelon was grafted to control soil pathogens in Asia in the early twentieth century (Sakata et al., 2005). Grafting was therefore first adopted in vegetable production to cope with soil-borne diseases. Since this time, vegetable grafting has also been used to enhance abiotic stress tolerance and water- and nutrient-use efficiency, increase yields, and improve fruit quality (Albacete et al., 2015; Colla et al., 2010; Lee, et al., 2010; Louws et al., 2010; Rouphael et al., 2010; Savvas et al., 2010; Schwarz et al., 2010). As the benefits of vegetable grafting have become increasingly well documented, this practice has spread worldwide and become especially popular in cucurbit and solanaceous crops in Asia and Europe (Lee et al., 2010). In the U.S., grafting has only recently begun to be adopted by vegetable growers. There is an increasing interest in this process, however, driven primarily by the need to cope with biotic and abiotic stresses, problems that have been exacerbated by recent restrictions on the use of methyl bromide (Kubota et al., 2008).

Tomato (*Solanum lycopersicum*) currently represents the largest number of grafted vegetables used in the U.S. Grafting is a proven, effective strategy used to overcome many challenges in tomato production including diseases and abiotic stresses. Various tomato rootstocks have resistance against soil-borne tomato diseases which result in significant yield

losses, including bacterial wilt, Fusarium wilt and Fusarium crown rot, Corky root rot, Verticillium wilt, and root-knot nematodes (Rivard and Louws, 2008; Rivard et al., 2010, 2012). Additionally, some rootstocks have been bred to be vigorous, so that they can tolerate abiotic stresses (temperature extremes, salinity, flood, etc.) (Schwarz et al., 2010) and enhance water- and nutrient- use efficiency (Djidonou et al., 2013, 2015). Grafting, therefore, may provide an effective technique to improve tomato production constrained by biotic and abiotic stresses while producing customer-preferred fruiting cultivars. Because of this, grafting is of interest to a broad spectrum of tomato producers including from organic or conventional, small or large, open field or protected production systems. Currently, however, grafting has not been widely adopted by tomato producers, and has been confined primarily to greenhouse production facilities.

The currently limited employment of grafted tomatoes in production systems across the U.S. is the result of the scarcity of knowledge on how to best prepare grafted tomatoes as well as how to maximize production from the resulting grafted plants. Specific difficulties of grafted tomato propagation and production are summarized below.

1) *Selection and preparation of rootstock and/or scion seedlings for grafting*: Grafted tomato propagators are challenged by the lack of information on the precise traits exhibited by specific tomato rootstocks and scions. The 60 tomato rootstock cultivars commercially available in 2016 (Kleinhenz and Short, 2016) can be grafted to the large number of consumer-preferred fruiting cultivars (scion) to potentially generate thousands of unique rootstock-scion combinations. The limited amount of information available regarding the seedling growth and graft performance of specific rootstock and scion cultivars hinders grafting operations by making it difficult to efficiently match rootstocks and scions to obtain optimal production.

2) *Optimization of healing conditions for grafted tomato*: In addition to the difficulties encountered in selecting rootstocks and scions for grafting, tomato propagators are also challenged by the lack of knowledge on optimal practices for the rapid and complete healing of newly grafted seedlings. Research-based information about the effects of key environmental

factors (light, temperature) on graft healing has started to emerge (Lee et al., 2016; Muneer et al., 2015; Nguyen et al., 2014; Vu et al., 2014a, 2014b), but extensive studies are still required to determine the optimal environmental conditions needed to maximize the efficiency of grafted seedling propagation.

3) *Management of grafted tomato production*: Grafted tomato users are challenged by the limited amount of information available on how to manage grafted plant production and maximize the benefits of grafting. While some reports have suggested that grafting improves tomato production (Khah et al., 2006; Savvas et al., 2009), other studies found that grafting does not necessarily have beneficial effects on plant growth, yield, and/or fruit quality. A careful reading of these studies indicates that the effects of grafting may be either advantageous or disadvantageous depending on the rootstock-scion combinations as well as the production conditions and management (Brajovic et al., 2012; Davis et al., 2008; Leonardi and Giuffrida, 2006; Rouphael et al., 2010). In short, more testing is needed to better define the performance of different rootstock-scion combinations under various conditions and management regimens, in order to allow growers select the optimal rootstock-scion combination and management for their region and limitations (abiotic stress conditions, pathogens common to the area, etc.).

The followings chapters describe studies designed to address the current challenges of grafted tomato propagation and production described above.

Chapter 2 described the development of an improved method for estimating vegetable seedling vigor, which is important in grafting for documenting rootstock and scion traits and scheduling grafting operations. The study was also designed to test correlations between destructive and non-destructive measures of seedling growth and the effect of tomato rootstock and scion seedling vigor on graft success. Emergence and biomass accumulation and distribution of 18 tomato rootstock and five scion cultivars were monitored in the greenhouse through 18 d after sowing using seven destructive and non-destructive measures; growing conditions were also monitored. Plant and environmental data were used: 1) to develop cultivar growth curves, rank-

sum values, and multi-component seedling vigor values and 2) to test correlations between percent canopy cover and other foliar measures. Also, seedlings representing all ninety rootstock-scion combinations and their associated seedling vigor values were cleft-grafted using accepted methods and grafted-plant survival was evaluated 2 weeks later. Overall seedling vigor and its components differed significantly between runs of the experiment and among cultivars, although most cultivars had similar rankings (relative vigor) in both runs. Rank-sum and seedling vigor values ordered cultivars similarly. Correlations between destructive and non-destructive measures were significant. Graft success did not differ among varietal combinations. We conclude: 1) that the tomato seedling vigor is genetically predisposed but environmentally modulated, differing widely among cultivars, 2) that the method to estimate seedling vigor described here is useful in grafting and other contexts, 3) that non-destructive measures can substitute for some destructive ones, and 4) that graft success in tomato is unrelated to rootstock and scion seedling vigor, provided proper grafting and healing techniques and commercial cultivars are used. Appendix A included the on-farm evaluation of the 90 grafted combinations and showed variability among combinations and farms.

Chapter 3 focused on developing a method for evaluating the healing of grafted plants under light or dark conditions pre- and post-grafting. 'Celebrity' and 'Maxifort' tomato seedlings were transferred to environmentally controlled chambers maintained at 0 or 250 μmol/m^2/s for 5 d, cleft-grafted (or not), then placed in chambers maintained at 0 or 135 μmol/m^2/s for 9 d, creating a total of four light treatments. Plant growth (stem diameter, scion length, plant height) was measured on day 9; survival and dye movement were taken on days 3, 5, 7 and 9 after grafting. The movement of water and solutes root-shoot via the xylem was visualized by placing the de-rooted seedlings in a solution containing Erythrosin B (a water-soluble dye) for 15 min, then measuring the distance of stain travel within the stem. Continuous darkness both pre- and post-grafting for a total of 14 d led to the death of grafted plants; otherwise, grafted-plant survivorship was similar among all other light treatments. In contrast, plant growth and dye

movement varied among light treatments beginning 5 d after grafting. Overall, plant elongation and dye-travel distances were greater in plants exposed to 135 versus 0 μmol/m^2/s after grafting, regardless of pre-grafting light levels. The results indicate: 1) that larger plant elongation and dye movement under light exposure post-grafting may suggest the beneficial effects of light on growth resumption as well as vascular reconnection and functioning in grafted plants, 2) that familiar but, so far, underutilized measures capable of tracking the progress of graft healing will be useful in research, commercial propagation, and educational settings and 3) that light exposure post-grafting seemed to promote graft healing, which should be studied further in tomato.

Chapter 4 focused on investigating the effects of light intensity during the healing period on graft healing in terms of survival as well as the resumption of vascular connection and plant growth in tomato. Two studies with two repeats each were conducted in two healing chambers. 'Cherokee Purple'/'Maxifort' splice-grafted tomato seedlings were healed under four light intensities (5, 50, 150, 300 μmol/m^2/s photosynthetically active radiation) providing solely by LED fixtures (60% blue, 20% red, and 20% white). Survivorship was recorded, and vascular reconnection was monitored by dye movement through the graft union. Plant regrowth was monitored including plant height, scion length, stem diameter at the rootstock and scion, stem and leaf fresh and dry weight, leaf area, specific leaf area, and compactness. In general, survivorship 5 and 10 d after grafting, as well as dye movement and plant regrowth 5 d after grafting did not differ among treatments. Plant regrowth 10 and 17 d after grafting increased with the increase of light intensity, except for relative growth of plant height, scion length and scion diameter in repeat two in study two which were largest at 150 μmol/m^2/s. The increased plant regrowth under higher light intensities may be associated with light regulation of morphogenesis and/or increased photosynthesis, but this relationship needs further investigation. These results indicate that the common practice of maintaining darkness or dim light during the early phase of the healing period may not be the optimal light management for grafted-plant propagation. Exposure to high light intensity up to 300 μmol/m^2/s can benefit the growth resumption of grafted tomato seedlings.

Chapter 5 tested separate and interactive effects of temperature and light intensity on the resumption of plant growth of grafted tomato seedlings. Grafted 'Cherokee Purple'/'Maxifort' seedlings were healed under two temperature (25/20 and 30/25 °C, day/night) by two light intensity (50 and 150 μmol/m^2/s) conditions in study one and under three temperature (15/25, 25/25 and 35/25 °C, day/night) by two light intensity (150 and 300 μmol/m^2/s) conditions in study two. Survival and plant regrowth including plant height, scion length, rootstock and scion diameter, leaf and stem fresh and dry weight, leaf area, compactness, and specific leaf area were monitored 10 d after grafting. In study one, the temperature did not affect measured variables while light intensity did, and temperature-light interactions were significant in three out of the 12 variables. Survival was not different (above 90%) in all treatments, plant regrowth was promoted under 150 versus 50 μmol/m^2/s, and 30/25 °C under 150 μmol/m^2/s tended to achieve the greatest plant regrowth. In study two, the main and interactive effects of temperature and light intensity were significant on most variables. Survival was higher under the moderate temperature at 25/25 °C regardless of light intensities and at 35/25 °C under 150 μmol/m^2/s. The moderate temperature at 25/25 °C under the higher light intensity at 300 μmol/m^2/s achieved the largest aboveground dry weight and compactness among the tested combinations. We conclude that the common practice of grafted-plant propagation with dim or dark conditions needs to be reconsidered, provided temperature control is reliable. The appropriate range of temperature can be from 25/20 °C to 30/25 °C (day/night), and the appropriate range of light intensity can be extended to 300 μmol/m^2/s under 25/25 °C.

Chapter 6 studied the effects of fertilization and grafting on plant growth, yield and fruit quality of fresh market tomato in conventional, open field systems. Studies were conducted twice in 2013 and 2014 as a split-plot design with fertilization management as the main plot and grafting as the subplot. Two fertilization treatments (pre-plant fertilization only, pre-plant fertilization plus standard fertigation) and two commercial tomato rootstocks ('Maxifort' and 'Emperador') and one experimental line ('320') were included. 'BHN589' was used as the scion

and ungrafted control. Plant growth was monitored using destructive and non-destructive measures. Ripe fruits were harvested weekly 7 and 5 times in 2013 and 2014, respectively. Total and marketable fruit weight and number were measured, and average marketable fruit weight and marketable yield percentage were calculated. A subset of fruit was used for fruit quality analysis including °Brix, pH, and titratable acidity (TA). The pre-plant fertilization plus standard fertigation treatment increased plant growth in both years and enhanced yield in 2014 compared to the pre-plant fertilization only treatment. Grafted plants achieved larger aboveground vegetative biomass, leaf area, leaf nitrogen (NO_3-N) content and truss number than ungrafted plants in 2013, but the opposite was true in 2014. Regardless, yield tended to be greater in grafted plants than ungrafted ones in both years. °Brix was higher in fruits from ungrafted versus grafted plants in both years, and it was not affected by fertilization treatments. Fruit pH and TA had inconsistent trends among fertilization and rootstock treatments across years. The interaction between fertilization and grafting treatments was not significant for most variables. We conclude that plant performance is influenced by fertilization regimens and grafting. Grafted plants have a higher yield potential than ungrafted ones under both high and low rates of fertilization in conventional, open field fresh market tomato production, while fruit quality needs to be carefully monitored since a lower °Brix value was observed in fruits from grafted plants.

The results of these studies provide research-based information and techniques for grafted-tomato propagators and users. The information helps to facilitate a broader application of grafting to tomato production, and eventually, benefit the tomato industry.

Chapter 2: A New Method to Estimate Vegetable Seedling Vigor, Piloted with Tomato, for Use in Grafting and other Contexts

The manuscript based on this chapter has been accepted for publication in HortTechnology 26(6).

Introduction

Seed and seedling vigor influence horticultural operations significantly and much has been done to establish operational definitions of and methods to assess both. For many, the line between seed vigor and seedling vigor is the transition of new seedlings from hetero- to autotrophy, i.e., from relying on seed reserves to photosynthesis for growth. Global, standardized protocols for estimating seed vigor define it as the inherent potential of seed from different seed lots to develop normal seedlings rapidly and uniformly, and to tolerate biotic and abiotic stresses (Baalbaki et al., 2009). These protocols involve monitoring germination and early-stage seedling development for crop-specific periods but rarely beyond the initial expansion of radicle, hypocotyl, and cotyledon(s) (Marcos-Filho, 2015). Lengthier evaluations often signal an interest in seedling vigor, generally accepted as the capacity of seedlings to convert growth factors into biomass once they become autotrophic (Whalley et al., 1966). As such, seedling vigor assessment requires different approaches but, presumably, similar levels of standardization (at least in reporting). Much has been written about the value of seedling vigor (Hernández-Herrera et al., 2014; Rebetzke et al., 2014; Spielmeyer et al., 2007); still, assessments of it remain less structured than seed vigor protocols.

Vegetable grafting is one globally-significant enterprise likely to benefit from improved methods of estimating and reporting seedling vigor. Preparing, using, and evaluating grafted

vegetable plants interest horticulturists, researchers, and educators worldwide (Albacete et al., 2015; Lee et al., 2010). Grafted plants have out-yielded ungrafted ones, especially when abiotic or biotic stress is prevalent, in multiple regions (Colla et al., 2010; Louws et al., 2010; Savvas et al., 2010; Schwarz et al., 2010). Grafting has also been a research tool in areas such as breeding and plant physiology (Kiihl et al., 1977; Simons et al., 2007). Millions of grafted tomato plants representing dozens of rootstock-scion combinations are prepared annually by hand and with machine assistance with success rates often exceeding 90%. Still, additional information, including on seedling vigor and graft success, would benefit propagators and crop scientists.

Pre-sowing estimates of expected cultivar vigor can help schedule propagation operations. For example, stem diameter is among the most important indicators of grafting readiness since rootstock and scion seedling stem diameters must not only be within a certain range but also be similar at the time of grafting (Oda et al., 1993; Yetişir and Sari, 2004). Stem diameters increase with age but at unknown rates, particularly among rootstock cultivars. To create the desired number of graft-eligible seedlings, propagators currently repeat sowings and work to speed or slow seedling growth through environmental manipulation. Both approaches are difficult and resource-demanding and lower the efficiency of grafted-plant production. Well-founded estimates of seedling growth (vigor) would allow for sowing and grafting periods to be scheduled more reliably and, thereby, limit the number of mismatched or unusable seedlings and investments in environmental or cultural manipulation. It may also limit the need to sort seedlings by stem diameter immediately before grafting, which is common. In fact, matching seedlings at grafting is more difficult with some rootstocks. While hybrid scion cultivars tend to be products of intense breeding and selection schemes emphasizing consistency, rootstock cultivars may be less consistent in emergence and growth. Some rootstock cultivars are open-pollinated and products of screening pre-existing germplasm, with less breeding and selection (King et al., 2010). Components of rootstock phenotype may vary, challenging users.

Seedling vigor information can also assist in cultivar selection. First, the number of tomato rootstocks commercially available in the U.S. increased from six to 60 between 2010 and 2016 (Kleinhenz and Short, 2016). Rootstock cultivars continue to become available far more quickly than research-based information on their performance before or after grafting, especially relative to scion cultivars growers prefer. Leonardi and Romano (2002) cautioned against allowing this discrepancy to persist. Second, the number of commercial and hobbyist grafted-plant producers and range of conditions under which their plants are grown are also increasing. Propagators currently work to shorten intervals between seeding and shipping as one way to increase production efficiency and profit potential. Faster-growing cultivars assist in that objective but should be avoided if high vigor conflicts with other desirable traits, as may occur between rootstock and scion cultivars. Third, rootstock and scion seedling vigor may be related to grafted-plant vegetative and fruiting characteristics. Overall, estimates of cultivar vigor have obvious potential to increase the efficiency of grafting operations and the reliability of rootstock and scion selection.

When multiple traits are measured, a large number of cultivars can be compared using rank-sum approaches (Kleinhenz, 2003; Osborne and Simonne, 2002). These approaches rely on ranking cultivars for each trait and then summing the ranks to develop a single value for each cultivar or cultivar-site combination. However, their underlying mathematics prevent rank-sum approaches from quantifying cultivar relative seedling vigor reproducibly since the range of rank-sum scores fluctuates with the number of cultivars involved. This fluctuation creates study to study variation. Also, rank-sum approaches do not quantify absolute growth rates, which is essential when quantifying and expressing seedling vigor. A method lacking the pitfalls of rank-sum approaches will allow investigators and professional horticulturists to obtain and use estimates of seedling vigor more reliably and widely.

Therefore, the first objective of this study was to test a method for estimating seedling vigor that: a) incorporates plant and environmental variables and b) differentiates cultivars and

describes their responses to growing conditions early in development. Estimates based on this method will be useful in grafting and other contexts. Emergence and growth of 23 tomato cultivars (18 rootstocks, five scions) under different environmental conditions were recorded using destructive and non-destructive measurements and cultivar-specific seedling vigor values were calculated using a straightforward formula. The relative seedling vigor of each cultivar was also calculated using the rank-sum approach described earlier (Kleinhenz, 2003; Osborne and Simonne, 2002).

Secondarily, the study was also designed to test correlations between destructive and non-destructive measures of seedling growth and relationships between rootstock and scion seedling vigor and graft success. Per standard protocol, grafted plants were prepared using seedlings with similar stem diameters. However, the seedlings represented cultivars expected to differ in seedling vigor. A total of 90 rootstock-scion combinations representing all combinations of seedling vigor resulted from grafting the 23 cultivars. Albacete et al. (2015) saw a need to study rootstock and scion traits, including vigor, more thoroughly and to use new tools in the process.

Materials and Methods

Plant materials and growing conditions

Eighteen commercial rootstock and five scion tomato cultivars were selected using grower input and publicly available information. The 18 selected rootstock cultivars were nominated by growers in three states, represent 12 developers, and contain a range of advertised disease resistance traits. The five selected scion cultivars are hybrid and heirloom and round- and oblong-fruited types. The cultivars used in this study and their developers/distributors are listed in Table 1.

The study was conducted twice (Run 1, Feb.-Mar. 2014; Run 2, Mar.-Apr. 2014). Both runs employed a completely randomized design with cultivar as the treatment. They were

completed in an environmentally controlled greenhouse at the Ohio Agricultural Research and Development Center in Wooster, OH. Rootstock and scion seed was sown on the same date (27 and 28 Feb. 2014 in Run 1 and 28 Mar. 2014 in Run 2) in 96-cell trays (cut into two halves) with cells measuring 1.13-inch wide, 1.5-inch long, and 2.25-inch deep. Half-tray units were preloaded with growing medium (Pro-Mix® MP Mycorrhizae™ Organik™; Premier Tech, Rivière-du-Loup, Canada) then sown with 48 seed of a single cultivar (three half-trays per cultivar). All half-trays were placed on a capillary mat (Kapmat; Buffalo Felt Products Corp., West Seneca, NY) underlain by 4-mm thick plastic on elevated benches in the greenhouse. Environmental conditions were monitored hourly throughout the study using data loggers (Hobo ProV2, version 2.5.0; Onset Computer Co., Pocasset, MA) and an Argus automatic control system (Argus Control Systems Ltd., Surrey, Canada). The daily averages of the recorded conditions in Run 1 and 2, respectively, were 23.2 and 23.7 °C temperature, 32% and 48% relative humidity, and 9.1 $mol \cdot m^{-2} \cdot d^{-1}$ and 14.8 $mol \cdot m^{-2} \cdot d^{-1}$ daily light integral (DLI) supplied by sunlight, 1000-W metal halide lamps (Multi-Vapor®; GE Lighting, East Cleveland, OH) and 1000-W high pressure sodium lamps (Ultra Sun®; Sunlight Supply, Vancouver, WA). Trays were hand-misted to wetness immediately after sowing; a bench-top, automated irrigation system was used thereafter to maintain soil moisture. Forty-four drippers (each with a flow rate of 1.5 gal/h) were distributed evenly among the trays on the capillary mat; each pulsed on for 10 min at 0600, 0900, 1200, 1500, 1800, and 2100 $_{HR}$. Emitters were supplemented by seven foggers (each with a flow rate of 8.1 gal/h) that pulsed on for 10 s every 15 min. Supplemental fertilization and pest and disease management were not applied.

Experimental design and data collection

Experimental units (replicates) within each experimental run consisted of a single half-tray sown with 48 seed of one cultivar. A total of three half-trays of each cultivar by 23 cultivars were prepared for each experimental run. All replicates were used for non-destructive measures

and two were used for destructive measures. The three half-trays (replicates) of each cultivar were distributed randomly on a 5.4 m x 1.8 m bench within the greenhouse room.

Emergence was determined by the presence of a hypocotyl hook above the surface of the rooting medium. Emergence counts were recorded daily from day 4 to 14, and day 4 to 13 after sowing in Run 1 and 2, respectively (beginning with the appearance of at least one hypocotyl hook and concluding when counts did not increase for two consecutive days for all cultivars).

Non-destructive canopy analysis was completed using an approach similar to that reported earlier (Bumgarner et al., 2012). Digital images collected with a tripod-mounted camera (Powershot A2000; Canon USA, Lake Success, NY) situated plumb 0.5 m over the center of a half-tray were analyzed with WinCAM software (Regent Instruments, Ville de Québec, Canada). Images of three half trays of each cultivar were collected on days 12, 15, and 18 and days 9, 12 and 15 after sowing in Run 1 and 2, respectively. WinCAM separated target colors (canopy) from background and calculated the percentage of the area bounded by the half tray associated with specific colors (percent canopy cover). The raw percent canopy cover value was then divided by the number of emerged seedlings in the half tray at the time of image capture to provide an adjusted percent canopy cover value.

Three representative plants were harvested from each unit (replicate) on days 12, 15 and 18 after sowing in both experimental runs. Seedling stems were cut at the rooting medium surface followed by five measures: a) plant height from the cut surface to apical meristem measured by a ruler (centimeter), b) aboveground fresh weight by a balance (milligram; MS3002S Precision Balance; Mettler Toledo, Greifensee, Switzerland), c) stem diameter 1 cm below the cotyledons by a digital caliper (millimeter; Traceable®; Control Company, Friendswood, TX), d) leaf area by an area meter (square centimeter; LI-3100C; LI-COR Biosciences, Lincoln, NE), and e) aboveground dry weight by a balance (milligram; MS3002S Precision Balance; Mettler Toledo) after drying in an oven (Fisher Scientific™ Isotemp™; Fisher Scientific, Waltham, MA) at 50 °C for 2 d.

Plants representing 90 rootstock-scion combinations (18 rootstocks x five scions) were cleft-grafted as outlined previously (Bumgarner and Kleinhenz, 2013) when they reached 1.5 to 2.5 mm in stem diameter. Not knowing the vigor of the cultivars used before sowing but expecting it to differ, scion and rootstock cultivars were sown on multiple days. This approach allowed us to select seedlings containing similar stem diameters -- but representing cultivars having different seedling vigor values -- when grafting all 90 rootstock-scion combinations. Immediately after grafting, plants were placed in a healing chamber located in the experimental greenhouse room and constructed using a polyvinyl chloride (PVC) frame covered by single layers of clear plastic sheeting and black knitted shade cloth (50% photosynthetic active radiation transmission; Tek Inc., Janesville, WI). Moisture was maintained using the same type of irrigation system as described before; however, this system was placed inside the chamber and contained 48 automated drippers (each with a flow rate at 1.5 gal/h) that pulsed on for 15 min at 0300 and every hour from 0600 to 2100 $_{HR}$, and six foggers (each capable of delivering 8.1 gal/h) pulsed on for 10 s every 15 min. The average temperature and relative humidity in the healing chamber was 22.8 °C and 87% in Run 1 and 23.3 °C and 88% in Run 2, respectively. Two weeks after grafting, graft survival was rated using an approach described earlier (Johnson and Miles, 2011); plants with a completely wilted scion were rated as dead and all others were rated as living (successful grafts).

Data analysis

The cumulative number of emerged seedlings as a percentage of the final count was fit to a three-parameter sigmoid model using SigmaPlot (version 12.5; Systat Software, San Jose, CA). Using estimated parameters provided by the model, the number of days to reach 90% of final emergence (T_{90}) were calculated in Microsoft Excel (2010; Microsoft Co., Redmond, WA). All calculated days smaller than four were adjusted to four, since emergence started 4 d after sowing.

Statistical analyses completed in SAS (version 9.3; SAS Institute, Cary, NC) included replication as a random effect, explored the separate and interactive effects of cultivar and run on

dependent variables, and were performed with by-run and pooled data. The GLIMMIX procedure and its LSMEANS statement with LINES and BYLEVEL options provided analyses of variance and multiple comparisons among cultivars (the latter with alpha = 0.05). Leaf area, stem diameter, and aboveground dry weight data recorded 12, 15, and 18 d after sowing were fit to a linear model using Proc Reg in SAS and to a quadratic model using the analysis options in SigmaPlot.

Correlations between non-destructive (percent canopy cover analyzed from digital images) and destructive measures (aboveground fresh and dry weight, stem diameter, leaf area, and plant height) were calculated using replicate data and the CORR procedure in SAS.

Further, seedling vigor values were calculated for each cultivar using a formula including four plant and two environmental variables and one constant:

$$\text{Vigor} = \frac{\text{aboveground dry weight (mg)} \times \text{stem diameter (mm)} \times \text{leaf area (cm}^2\text{)} \times (1 \times 10^5)}{(T_{90} \times \text{GDD} \times \text{DLI})}$$

Where T_{90} represents days to reach 90% of final emergence; where all biomass values are measures taken 18 d after sowing; where growing degree days (GDD) and DLI represent these variables accumulated by 18 d after sowing; and where daily GDD is calculated using a base and ceiling temperature of 10 and 27 °C, respectively.

A rank-sum approach after Kleinhenz (2003) and Osborne and Simonne (2002) was also used to compare the relative seedling vigor of the cultivars. Briefly, cultivars were ranked based on their mean value of each of the individual plant-based variables also used in calculating vigor as described above. Then, individual rank values were summed to create a single value for each cultivar.

Results

Mean values of all measured plant variables differed between experimental runs (Table 2). Overall, seedlings grew faster in Run 2 than in Run 1 but at cultivar-specific rates. Run by cultivar interactions were significant for all variables except for emergence T_{90}. As an example,

mean aboveground dry weight values of ‘Arnold’ and ‘B.B.’ 18 d after sowing were nearly 1.9 and 3.5 times greater in Run 2 than in Run 1, respectively (Table 3). Ordering the 23 cultivars based on mean aboveground dry weight showed that the positions of six cultivars differed by more than five places between runs while the rank of other cultivars changed less dramatically.

As expected, cultivar had a significant ($P < 0.0001$) effect on all seedling emergence and growth variables in pooled and by-run analyses of variance and in mean separation tests (Tables 2-3). The majority of cultivars displayed consistent levels of relative growth between experimental runs. For example, ‘Trooper’, ‘Aooni’, and ‘Estamino’ emerged most slowly, requiring 8.7 to 10.1 d to reach emergence T_{90} in Run 1 and 7.9 to 8.7 d in Run 2. ‘Arnold’, ‘Beaufort’, ‘Kaiser’, ‘Maxifort’ and ‘Stallone’ emerged most rapidly, reaching emergence T_{90} by 6.1 d in Run 1 and 5.3 d in Run 2. Emergence rates of other cultivars were intermediate, with T_{90} values ranging from 5.8 to 8.0 in Run 1 and 5.4 to 7.7 in Run 2. Emergence T_{90} values of the five scion cultivars were intermediate and relatively more stable than those of the 18 rootstock cultivars; scion T_{90} values registered 5.5 to 7.0 in both runs. Similar trends were evident in other variables. In Run 1, ‘Beaufort’ had the highest percent canopy cover, ‘Arnold’ had the largest leaf area, aboveground fresh and dry weight, and stem diameter, and ‘Maxifort’ had the largest plant height. In Run 2, ‘Kaiser’ and ‘Maxifort’ were among the cultivars displaying the fastest growth. ‘Trooper’ had the lowest mean values of all growth variables in both experimental runs.

Individual cultivar seedling vigor values (Table 4) ranged from 3 (‘Trooper’) to 1727 (‘Arnold’), with a mean, median and standard deviation of 304, 190, and 382 in Run 1; from 145 (‘Trooper’) to 11504 (‘Kaiser’), with a mean, median and standard deviation of 2314, 1593, and 2454 in Run 2, respectively. Plotting vigor values revealed that their distribution was skewed, with most values below the midpoint of the range (865 in Run 1 and 5824 in Run 2) and a smaller number of cultivars displaying values well above-average. The two lowest and highest vigor values belonged to rootstock cultivars. Seedling vigor values for most cultivars relative to other

cultivars were similar in both runs. However, rankings based on vigor differed by more than five places between runs for five cultivars.

Study-wide rank-sum values ranged from 4 to 92 across all cultivars (data not shown), a narrower range than found for seedling vigor (Table 4). Seedling vigor values (3-11504) and summed rank scores (4-92) ordered the 23 cultivars similarly low-high in terms of emergence and seedling growth rates.

Correlations between direct (destructive) and indirect (non-destructive) assessments of seedling vigor were strong (Table 5). WinCAM-mediated measures of canopy cover calculated from digital images were significantly (r^2 = 0.47-0.95) related to direct measures of aboveground fresh and dry weight, stem diameter, and leaf area in both runs and among rootstock and scion cultivars. Relationships between percent canopy cover and direct measures of plant height were inconsistent but significant on days 18 and 15 after sowing in Run 1 and Run 2, respectively.

Leaf area, stem diameter, and aboveground dry weight values displayed significant ($P <$ 0.001) linear and quadratic tendencies (Figure 1; linear data not shown). Quadratic r^2 values ranged from 0.63 to 0.94 for leaf area, 0.64 to 0.94 for stem diameter, and 0.50 to 0.71 for aboveground dry weight. Within each run, overall rootstock and scion regression lines were similar in shape and placement. Rootstock and scion lines retained their shape across runs.

Graft survivorship among the 90 rootstock-scion combinations ranged from 92% to 100% and did not differ significantly among combinations (data not shown).

Discussion

Seedling vigor differed between experimental runs but to extents depending on cultivars (Table 2-3). The daily average of recorded relative humidity and the total accumulated GDD and DLI 18 d after sowing was 32%, 257 and 172 mol·m^{-2} and 48%, 262 and 282 mol·m^{-2} in Run 1 and 2, respectively. Total accumulated DLI was similar between runs through the first 7 d after sowing but not over the remaining 11 d of each study period. Plants received 2.25 times more

total accumulated DLI over days 8-18 in Run 2 than in Run 1. Growth responses to this difference were cultivar-specific and expected given previous reports of tomato emergence and seedling growth rates (Gent, 1986; Gogo et al., 2012; Hussey, 1963, 1965). Although the majority of cultivars reached T_{90} faster and grew more rapidly in Run 2 than Run 1, several cultivars displayed the opposite behavior. While growth differed more widely within the rootstock versus the scion cultivar group, the shapes of rootstock and scion growth curves were similar (Figure 1). Cultivars grown from pelleted and primed seed (except for 'Shield') tended to have larger seedling vigor values. Fully testing the effects of priming, pelleting, or other seed treatments on seedling vigor was beyond the scope of this study. However, the process of estimating seedling vigor described here clearly differentiated cultivars and their growth responses to environmental conditions. We also expect it to be useful in differentiating other treatment effects.

Plant and environmental data can be used to calculate seedling vigor. Like other complex traits, seedling vigor has objective foundations but it is often evaluated subjectively. Here, we asked if a process for estimating seedling vigor could be established and used to compare cultivars or other experimental units, much like internationally recognized methods contribute to assessing and reporting seed vigor (Association of Official Seed Analysts, 2002; Hoffmaster et al., 2003; Sako et al., 2001). Data representing four plant and two environmental variables were used to calculate seedling vigor values for 23 tomato cultivars (Table 4). The vigor formula assigns equal weight to all input values. Also, its structure assures that changing one or more input values leads to an equal-sized change in vigor value. However, to verify this, we used a large speculative dataset encompassing wide ranges of the six input variables. Outcomes of the test (data not shown) clearly demonstrated that changing an input value(s) results in an equal, by percent, change in vigor value. So, the more values (variables) included in the calculation, the greater the potential range of vigor values and their sensitivity to plant physiology and growing conditions. Four plant variables were used here; however, using a smaller number along with environmental variables still could enhance the reporting of seedling vigor and investigators' abilities to compare results

among studies. Current reports vary widely in their description of growing conditions. Therefore, identifying trends in plant responses to growing conditions across studies can be difficult. A formula incorporating plant and environmental data helps standardize the description of plants' conversion of growth factors into biomass. High levels of standardization have been essential to fostering the widespread use of seed vigor tests and their results.

Nevertheless, improving the approach used in this study to obtain seedling vigor values that can be compared across studies will widen its application. For example, alternative methods could begin before emergence with measures taken in companion benchtop protocols. Likewise, methods could include measures of root biomass taken on seedlings after emergence. Regardless, seedling vigor values are likely to be useful in comparing across studies, especially when relative values are used and growing conditions are clearly reported. Understanding seedling vigor more completely may also help explain the vegetative and fruiting characteristics of rootstock and scion combinations, which clearly vary (e.g., Davis et al., 2008; Leonardi and Giuffrida, 2006), but for reasons that remain largely unknown (Albacete et al., 2015).

When multiple traits are measured, a large number of cultivars can also be compared using rank-sum approaches (Kleinhenz, 2003; Osborne and Simonne, 2002). Here, study-wide rank-sum values registered 4 to 92 across all cultivars (data not shown), a narrower range than for seedling vigor which equaled 3 to 11504 (Table 4). Rank-sum and seedling vigor values ordered the 23 cultivars similarly low-high in terms of emergence and seedling growth rates. However, seedling vigor values are more sensitive to variations in plant and environmental data and, therefore, more useful in research and commercial settings.

Seedling growth is tracked with destructive measures, machine vision systems (Conrad, 2004; Giacomelli et al., 1996), and plant image analysis (Bumgarner et al., 2012). Image analysis may complement or reduce the need for destructive sampling if data obtained from digital images are significantly correlated with data from direct, destructive measures. However, correlations decline with canopy closure when leaves overlap and are under-represented in image analysis

(Lin et al., 2002). In this study, WinCAM software-mediated estimates of canopy size based on digital images correlated significantly with direct measures of aboveground fresh and dry weight, leaf area, stem diameter, and, less often, plant height when tested 12, 15 and 18 d after sowing (Table 5). Therefore, rapid and inexpensive non-destructive measures early in plant growth have applications in research and production settings.

These results are important whenever large numbers of tomato cultivars are sown, especially if the goal is to graft them. Graft success is more likely when stem diameters are similar (Oda et al., 1993; Pofu et al., 2013; Yetişir and Sari, 2004), especially when machines are involved. Optimizing the process demands seedlings to be ready for grafting simultaneously. Using data and equations depicted in Figure 1B but with more time points, we can calculate the number of days seedlings of individual cultivars may have required to reach 1.5 mm in stem diameter, the generally accepted minimum for grafting. Expected windows of graft eligibility (1.5 to 3.0 mm stem diameter) could be calculated from similar datasets provided they were developed using more time points and over a sufficient period of time (regression in Figure 1B terminated at approximately 1.5 and 2.5 mm stem diameter in Run 1 and 2, respectively).

All 90 rootstock-scion combinations had at least 92% survival 14 d after grafting. High rates of graft success are expected in tomato (Johnson and Miles, 2011) when proper techniques (e.g., pairing rootstock and scion seedlings by stem diameter) are used. Data reported here suggest that graft success rates are unaffected by relative rootstock and scion seedling vigor, provided accepted grafting and healing techniques and commercial cultivars are used.

We conclude: 1) that tomato seedling vigor is genetically predisposed but environmentally-modulated, differing widely among cultivars; 2) that seedling vigor can be estimated reliably and reproducibly with plant and environmental data; 3) that direct and indirect measures of selected plant traits are strongly correlated, enhancing opportunities to employ rapid and inexpensive non-destructive measures in research and production settings; 4) that new tools, such as growth curves and seedling vigor values, can assist in cultivar and other comparisons and

in scheduling grafting and other operations; and 5) that graft success in tomato is unrelated to rootstock and scion seedling vigor, provided accepted grafting and healing techniques and commercial cultivars are used.

Table 1. List of 18 commercial tomato rootstock and five scion cultivars used in this study for documenting their seedling vigor

Rootstock cultivar[z]	Seed company/ distributor	Rootstock cultivar	Seed company/ distributor	Scion cultivar[y]	Seed company/ distributor
Aiboh	Asahi Industries (Arakawa-Ku, Japan)	Kaiser	Rijk Zwaan (De Lier, Netherlands)	Brandywine	NE Seed (Hartford, CT)
Akaoni	Asahi Industries	Maxifort	DeRuiter Seeds	Better Boy	NE Seed
Aooni	Asahi Industries	Resistar	Hazera Seeds (Berurim, Israel)	Celebrity	NE Seed
Armada	Takii Seed (Kyoto, Japan)	RST-04-105	DP Seeds (Yuma, AZ)	Cherokee Purple	NE Seed
Arnold	Siegers Seed Co. (Holland, MI)	RST-04-106	DP Seeds	San Marzano 2	NE Seed
B.B.	Takii Seed	Shield	Rijk Zwaan		
Beaufort	DeRuiter Seeds (Cambourne, United Kingdom)	Stallone	Rijk Zwaan		
Cheong Gang	Seminis Vegetable (St. Louis, MO)	Supernatural	A.P. Whaley Seeds (Mount Horeb, WI)		
Estamino	Enza Zaden (Enkhuizen, Netherlands)	Trooper	Seedway (Elizabethtown, PA)		

[z] Seed of only 'Kaiser' and 'Stallone' was pelleted; all other seed was not pelleted. Seed of only 'Arnold', 'Beaufort', 'Kaiser', 'Maxifort', 'Shield', and 'Stallone' was primed; all other seed was not primed.
[y] 'Celebrity' is determinate; the other four scions are indeterminate.

Table 2. *P* values of Type III tests of the effects of cultivar and run on seedling emergence and growth variables of 18 tomato rootstock and five scion cultivars as recorded in a greenhouse in Wooster, OH in Feb.-Apr. 2014

Source of variance	Emergence T_{90}[z]	Canopy cover	Leaf area	Aboveground fresh wt	Aboveground dry wt	Plant ht	Stem diam
Cultivar	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Run	0.05	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cultivar x Run	0.2	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cultivar (Run 1)[y]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cultivar (Run 2)[y]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[z] T_{90} represents days to reach 90% of final emergence.
[y] Run 1 (27 Feb.-17 Mar. 2014); Run 2 (28 Mar.-15 Apr. 2014).

Table 3. Seedling emergence and growth variables 18 d after sowing of 18 tomato rootstock and five scion cultivars in a greenhouse in Wooster, OH in Feb.-Apr. 2014

Cultivar[z]	T_{90}[y]	Canopy cover (%)	Leaf area (cm^2)[x]	Aboveground fresh wt (g)[w]	Aboveground dry wt (mg)[v]	Plant ht (cm)[u]	Stem diam (mm)[t]
			Run 1 (27 Feb.-17 Mar. 2014)				
Aiboh	6.8 de[s]	58 gh	10 h	0.31 h-k	21 f-h	4.27 b-g	1.37 e-g
Akaoni	6.0 fg	64 e-h	10 gh	0.26 jk	14 h	3.52 hi	1.29 e-g
Aooni	8.7 b	59 gh	13 e-g	0.37 e-i	21 f-h	3.97 d-h	1.34 e-g
Armada	6.1 fg	67 d-h	15 ef	0.39 d-h	24 e-g	3.67 gh	1.48 c-e
Arnold	4.0 i	88 ab	29 a	0.80 a	57 a	4.38 a-e	1.81 a
B.B.	5.8 g	72 c-f	15 ef	0.42 d-g	26 d-f	3.92 e-h	1.47 c-e
Beaufort	4.2 i	96 a	21 bc	0.63 b	50 a	4.58 a-d	1.71 ab
Better Boy	6.1 fg	63 e-h	12 f-h	0.35 f-j	25 e-g	3.95 e-h	1.38 d-g
Brandywine	6.2 fg	66 e-h	12 e-h	0.37 e-i	22 f-h	3.72 f-h	1.38 d-g
Celebrity	7.0 d	74 c-e	13 e-h	0.35 f-j	22 fg	4.03 c-h	1.38 d-g
Cheong Gang	6.2 fg	65 e-h	16 de	0.45 c-f	23 fg	4.70 ab	1.39 d-g
Cherokee Purple	5.9 fg	62 f-h	14 ef	0.45 c-f	28 c-f	4.65 a-c	1.47 c-e
Estamino	9.2 b	61 f-h	13 e-h	0.24 k	18 gh	2.95 ij	1.09 h
Kaiser	5.1 h	87 ab	23 b	0.48 cd	36 bc	3.50 hi	1.36 e-g
Maxifort	4.8 h	88 ab	20 bc	0.53 bc	41 b	4.93 a	1.56 b-d
Resistar	6.9 de	78 b-d	19 cd	0.53 bc	32 c-e	3.93 e-h	1.56 b-d
RST-04-105	8.0 c	64 e-h	13 e-h	0.32 g-k	21 f-h	2.75 j	1.26 f-h
RST-04-106	6.2 fg	72 c-f	14 ef	0.43 c-g	25 e-g	4.02 d-h	1.45 d-f
San Marzano 2	6.4 ef	69 d-g	15 d-f	0.44 c-f	31 c-e	4.23 b-g	1.65 a-c
Shield	7.1 d	55 h	10 H	0.29 i-k	17 gh	3.53 hi	1.23 gh
Stallone	6.1 fg	83 bc	20 bc	0.47 c-e	33 b-d	4.32 a-f	1.39 d-g
Supernatural	7.7 c	59 gh	13 e-h	0.35 f-j	26 d-f	4.32 a-f	1.41 d-g
Trooper	10.1 a	34 i	4 i	0.06 l	4 i	1.92 k	0.78 i
P	*0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*

Continued

Table 3 continued

Cultivar[z]	T_{90}[y]	Canopy cover (%)	Leaf area (cm^2)[x]	Aboveground fresh wt (g)[w]	Aboveground dry wt (mg)[v]	Plant ht (cm)[u]	Stem diam (mm)[t]
			Run 2[r] (28 Mar.-15 Apr. 2014)				
Aiboh	6.1 e-j	45 fg	20 fg	0.61 ij	51 g-i	5.53 i	1.84 gh
Akaoni	7.2 b-e	61 a-e	44 cd	1.38 b-f	108 b-e	8.60 a-c	2.42 b-e
Aooni	8.4 ab	50 e-g	29 d-g	0.88 f-i	65 e-i	7.98 b-e	1.91 g
Armada	6.2 d-i	65 a-d	39 cd	1.74 ab	121 b-d	8.73 a-c	2.81 ab
Arnold	4.0 k	61 a-e	43 cd	1.13 d-i	108 b-f	7.25 c-g	2.26 c-g
B.B.	6.0 e-j	67 a-c	40 cd	1.48 b-e	90 c-h	9.80 a	2.81 ab
Beaufort	4.2 k	73 ab	36 c-e	0.83 g-j	83 c-i	6.48 e-i	2.16 d-g
Better Boy	6.8 c-h	53 d-g	31 d-g	1.03 e-i	83 c-i	7.20 c-h	2.05 e-g
Brandywine	6.2 d-i	55 c-f	32 d-g	1.10 d-i	67 e-i	7.52 c-g	2.41 b-f
Celebrity	6.9 c-g	51 e-g	36 c-e	1.03 e-i	78 d-i	6.98 d-i	2.21 c-g
Cheong Gang	5.4 g-k	60 b-e	39 cd	1.21 c-g	93 c-g	8.40 a-d	2.23 c-g
Cherokee Purple	5.5 f-k	43 fg	19 fg	0.67 h-j	44 hi	6.68 e-i	1.85 gh
Estamino	7.9 a-c	62 a-e	34 c-f	0.75 g-j	52 g-i	7.18 c-h	1.87 gh
Kaiser	4.6 jk	74 a	76 a	2.12 a	185 a	9.83 a	2.62 a-c
Maxifort	5.3 h-k	74 a	61 ab	1.61 a-d	146 ab	9.32 ab	2.56 a-d
Resistar	6.0 e-j	61 a-e	30 d-g	0.75 g-j	57 g-i	6.40 e-i	2.26 c-g
RST-04-105	7.7 a-d	56 c-f	44 cd	1.04 e-i	78 d-i	6.33 f-i	2.34 c-f
RST-04-106	6.6 c-i	53 d-g	39 cd	1.16 d-h	84 c-i	7.90 b-f	2.43 b-e
San Marzano 2	6.5 c-i	41 g	49 bc	1.71 a-c	128 bc	8.35 a-d	2.95 a
Shield	6.8 c-h	68 a-c	22 e-g	0.65 h-j	46 g-i	5.97 g-i	1.98 fg
Stallone	5.2 i-k	72 ab	41 cd	1.16 d-h	109 b-e	7.65 c-f	2.39 b-f
Supernatural	7.0 b-f	46 fg	23 e-g	0.66 h-j	60 f-i	5.60 hi	1.90 g
Trooper	8.7 a	26 h	17 g	0.34 j	37 i	2.45 j	1.46 h
P	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*

Continued

Table 3 continued

[z] Five cultivars in bold are scions (‘Better Boy’, ‘Brandywine’, ‘Celebrity’, ‘Cherokee Purple’, and ‘San Marzano 2’). The other 18 cultivars are rootstocks. ‘Kaiser’ and ‘Stallone’ seed was pelleted. ‘Arnold’, ‘Beaufort’, ‘Kaiser’, ‘Maxifort’, ‘Shield’ and ‘Stallone’ seed was primed.

[y] T_{90} represents days to reach 90% of final emergence.

[x] 1 cm^2 = 0.1550 $inch^2$.

[w] 1 g = 0.0353 oz.

[v] 1 mg = 3.5274 x 10-5 oz.

[u] 1 cm = 0.3937 inch.

[t] 1 mm = 0.0394 inch.

[s] Means within the same column in each run followed by the same letter are not significantly different ($P < 0.05$) as analyzed using the LINES and BYLEVEL options in the LSMEANS statement in the GLIMMIX procedure (SAS version 9.3; SAS Institute, Cary, NC).

[r] Percent canopy cover in Run 2 was measured 15 d after sowing.

Table 4. Seedling vigor values calculated for 18 tomato rootstock and five scion cultivars in a greenhouse in Wooster, OH using a formula including four plant and two environmental variables and one constant

Cultivar[z]	Vigor[y]			
	Run 1 (27 Feb.-17 Mar. 2014)		Run 2 (28 Mar.-15 Apr. 2014)	
Aiboh	106	f-h[x]	401	c
Akaoni	72	gh	2292	bc
Aooni	102	f-h	878	bc
Armada	198	e-h	3554	bc
Arnold	1727	a	4008	bc
B.B.	230	e-h	2218	bc
Beaufort	1024	b	2437	bc
Better Boy	154	e-h	1593	bc
Brandywine	130	e-h	1134	bc
Celebrity	141	e-h	1316	bc
Cheong Gang	190	e-h	2256	bc
Cherokee Purple	234	e-h	393	c
Estamino	65	h	606	c
Kaiser	513	cd	11504	a
Maxifort	610	c	5244	b
Resistar	315	d-f	956	bc
RST-04-105	99	f-h	1557	bc
RST-04-106	191	e-h	1844	bc
San Marzano 2	305	d-g	3751	bc
Shield	73	gh	391	c
Stallone	357	de	4189	bc
Supernatural	154	e-h	544	c
Trooper	3	h	145	c
P	*<0.0001*		0.017	

[z] Five cultivars in bold are scions ('Better Boy', 'Brandywine', 'Celebrity', 'Cherokee Purple', and 'San Marzano 2'). The other 18 cultivars are rootstocks. 'Kaiser' and 'Stallone' seed was pelleted. 'Arnold', 'Beaufort', 'Kaiser', 'Maxifort', 'Shield' and 'Stallone' seed was primed.

[y] $\text{Vigor} = \frac{\text{aboveground dry weight (mg)} \times \text{stem diameter (mm)} \times \text{leaf area (cm}^2\text{)} \times (1 \times 10^5)}{(T_{90} \times \text{GDD} \times \text{DLI})}$

Where T_{90} represents days to reach 90% of final emergence; where all biomass values are measures taken 18 d after sowing; where GDD and DLI represent these variables accumulated by 18 d after sowing; and where daily GDD is calculated using a base and ceiling temperature of 10 and 27 °C, respectively. In this experiment and calculation, GDD and DLI were 257 and 172 $\text{mol}\cdot\text{m}^{-2}$ in Run 1, and 262 and 282 $\text{mol}\cdot\text{m}^{-2}$ in Run 2. 1 mg = 3.5274×10^{-5} oz; 1 mm = 0.0394 inch; 1 cm^2 = 0.1550 inch^2; (1.8 x °C) + 32 = °F.

[x] Means within the same column in each run followed by the same letter are not significantly different ($P < 0.05$) as analyzed using the LINES and BYLEVEL options in the LSMEANS statement in the GLIMMIX procedure (SAS version 9.3; SAS Institute, Cary, NC).

Table 5. The relationship between percent canopy cover based on digital image analysis and direct measures of seedling aboveground fresh and dry weight, stem diameter, leaf area, and plant height for 18 tomato rootstock and five scion cultivars grown in a greenhouse in Wooster, OH in Feb.-Apr. 2014

Correlation	Run 1[z]			Run 2[z]	
	Day 12	Day 15	Day 18	Day 12	Day 15
Percent canopy cover: Aboveground fresh wt	0.71[y] (0.0002)	0.80 (<0.0001)	0.86 (<0.0001)	0.76 (<0.0001)	0.65 (0.0009)
Percent canopy cover: Aboveground dry wt	0.47 (0.02)	0.84 (<0.0001)	0.88 (<0.0001)	0.79 (<0.0001)	0.73 (<0.0001)
Percent canopy cover: Stem diam	0.56 (0.005)	0.57 (0.005)	0.78 (<0.0001)	0.59 (0.003)	0.71 (0.0002)
Percent canopy cover: Leaf area	0.95 (<0.0001)	0.90 (<0.0001)	0.90 (<0.0001)	0.85 (<0.0001)	0.69 (0.0003)
Percent canopy cover: Plant ht	0.07 (0.8)	0.14 (0.5)	0.59 (0.003)	0.15 (0.5)	0.51 (0.01)

[z] Run 1 (27 Feb.-17 Mar. 2014); Run 2 (28 Mar.-15 Apr. 2014). Digital images were not taken on day 18 in Run 2.

[y] Pearson Correlation Coefficient (r^2) followed by probability value in parentheses. N = 23.

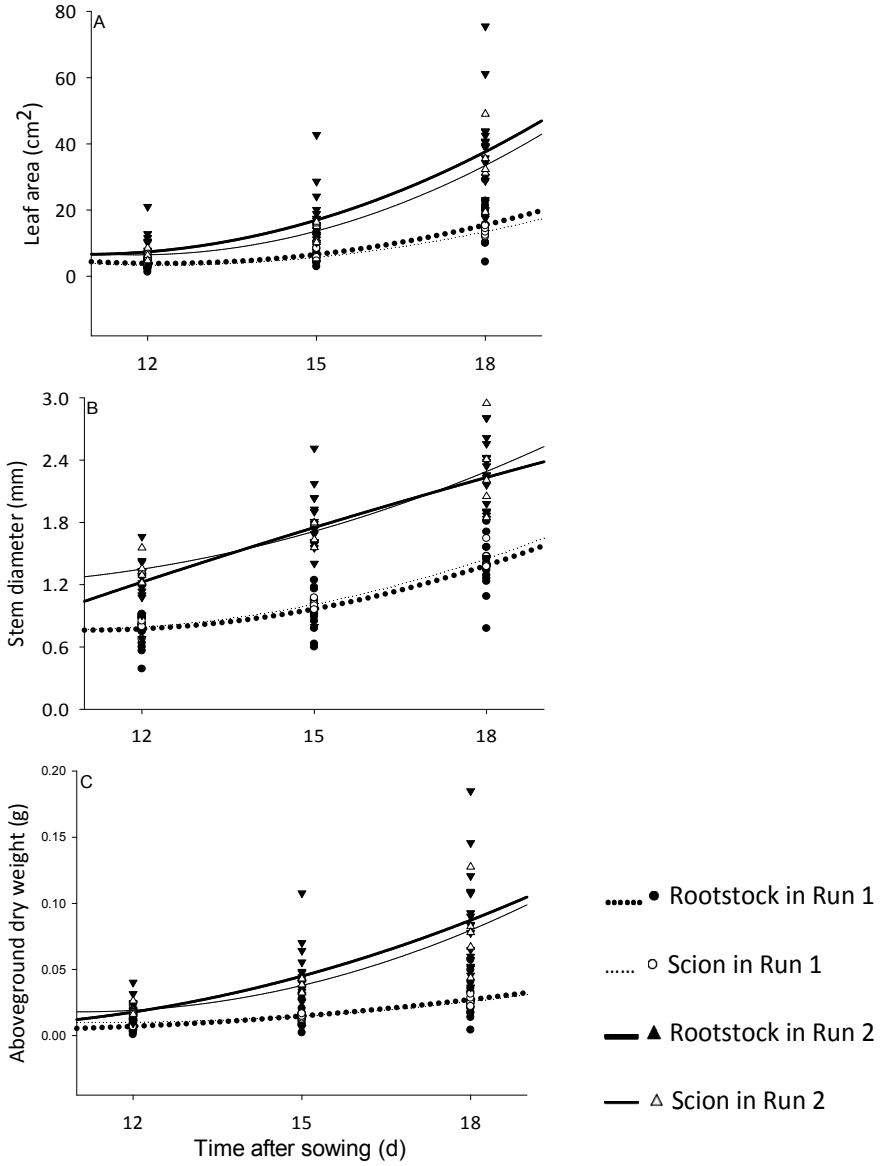

Figure 1. Quadratic regression of leaf area (A), stem diameter (B) and aboveground dry weight (C) of 18 tomato rootstock and five scion cultivars grown in a greenhouse in Wooster, OH. Run 1 (27 Feb.-17 Mar. 2014); Run 2 (28 Mar.-15 Apr. 2014). Each data point is the average of a cultivar on the specified day. 1 cm^2 = 0.1550 $inch^2$; 1 mm = 0.0394 inch; 1 g = 0.0353 oz

Chapter 3: Multiple Measures Reveal that Pre- and Post-Grafting Light Levels Influence the Healing Rate of Newly Grafted Tomato Plants

Introduction

Wounding triggers cascades of reactions in plants that are only partially understood (Howe, 2004). However, it is clear that wound healing demands energy (Tornbom and Oliveira, 1993) taken from stored reserves or recently fixed carbon.

Grafting involves purposeful but potentially lethal wounding. In grafting, functional root-shoot axes are severed as rootstock seedlings are decapitated (with their canopies discarded) and scion seedlings are de-rooted (with their root systems discarded). Next, when joined at the graft union, the two remaining portions must re-establish vascular connections to create a viable plant able to withstand potentially severe stresses during crop production. Graft union development involves elaborate signaling and cell proliferation and differentiation, which are regulated by endogenous and exogenous factors (Waard and Zaubin, 1983). Moreover, typically, vegetable seedlings are only 2-4 weeks old when grafted and scion leaves are often removed in the process (Masterson et al., 2016). Therefore, it is reasonable to ask how pre- and post-grafting conditions influence healing rates, perhaps as a result of their altering energy availability before and/or after grafting. Regardless, grafted vegetable plants are produced and used worldwide because they can overcome crop stresses, including root-borne diseases (King et al., 2008; Rivard et al., 2012), temperature and root zone moisture extremes (Nilsen et al., 2014; Schwarz et al., 2010), and salinity. Grafted plants have also demonstrated greater water- and nutrition-use efficiencies (Djidonou et al., 2013, 2015).

Anatomical, biochemical, and other studies have shown that graft healing begins with intercellular communication and callus proliferation at the interface of rootstock and scion tissues (Yin et al., 2012). Callus formation is followed by vascular differentiation, then secondary xylem and phloem development, and rootstock-scion connection (Fan et al., 2015; Martínez-Ballesta et al., 2010). Reestablishing fully-functional root-shoot communication and transport (perhaps first of water) are, presumably, prerequisites for additional growth and development. However, relatively few techniques have been employed to document the progression of root-shoot reconnection in grafted vegetable plants at its early stages and in relatively large numbers of plants subjected to different treatments before or immediately after grafting. A simple method of observing dye movement with the unaided eye to verify vascular reconnection and functioning will assist the evaluation of graft healing.

In fact, much remains to be learned about the healing of vegetable grafts, especially if the goal includes optimizing environments in which it occurs or utilizing vegetable grafting more fully in research, commercial, and educational settings. Slow or poor healing is particularly detrimental to commercial operations. Grafted-plant production becomes more resource-demanding (e.g., space, labor, water, environmental control) and low-quality grafted plants, if used, may underperform or even die (Errea et al., 1994). Therefore, identifying conditions that promote rapid healing and high-quality grafted plants is important.

The goal of this work was to enhance the understanding of pre- and post-grafting light treatment on grafted-plant survival and short-term growth (healing), partly by including measures that, so far, appear to have been underutilized in the vegetable graft-healing literature. Tomato seedlings and grafted plants were exposed to a total of four pre- and post-grafting light treatments. Plant condition was monitored through 9 d after grafting with a combination of survivorship ratings (Oda et al., 1993) and plant-based measures, including ones obtained by using a water-soluble dye to visualize the movement of water from roots to shoots, across the graft union. Reports (e.g., Chia and Kubota, 2010; Jang et al. 2011, 2013, 2014; Kim et al., 2005; Muneer et

al., 2015; Nguyen et al., 2014; Von Arnim and Deng, 1996) detail that light intensity and composition regulate plant morphology before grafting as well as photosynthesis, growth and graft-take after grafting in pepper, cucumber, and watermelon. However, fewer reports describing light effects on tomato healing are available and consistent conclusions are limited (Nobuoka et al., 1996; Nobuoka et al., 2005; Vu et al., 2014b). More important, related studies have not included both pre- and post-grafting light treatment or used measurements for healing evaluation collected here.

Materials and Methods

Seedling production

The experiment was repeated three times in 2013-2014 at the Ohio Agricultural Research and Development Center in Wooster, OH. 'Maxifort' (DeRuiter Seeds, Cambourne, United Kingdom) was used as the rootstock in standard cleft-grafting. 'Celebrity' (Johnny's Selected Seeds, Winslow, ME) was used as the scion in standard cleft-grafts, as the rootstock and scion in self-grafts, and as the ungrafted control.

It is imperative to graft seedlings having similar stem diameters. Therefore, 'Maxifort' and 'Celebrity' seeding dates were selected based on expected growing conditions and cultivar emergence and growth rates. Seeding dates were 4 Oct. 2013 ('Maxifort', 'Celebrity') in Repeat 1, 1 Nov. 2013 ('Celebrity') and 3 Nov. 2013 ('Maxifort') in Repeat 2, and 25 June 2014 ('Celebrity') and 26 June 2014 ('Maxifort') in Repeat 3. All seeds were sown in 200-cell plug trays filled with growing medium (Pro-Mix® MP Mycorrhizae™ Organik™; Premier Tech, Rivière-du-Loup, Canada). Trays were placed on a capillary mat (Kapmat; Buffalo Felt Products Corp., West Seneca, NY) in a greenhouse with temperature settings of 22-27 °C day/17-24 °C night. Sunlight was supplemented with 1000-W metal halide lamps (Multi-Vapor®; GE Lighting, East Cleveland, OH) and 1000-W high-pressure sodium lamps (Ultra Sun®; Sunlight Supply, Vancouver, WA) from dawn for 16 h unless sunlight exceeded 400 W/m^2 for 20 min in Repeat 1

and 2, or 350 W/m^2 in Repeat 3. Irrigation was applied manually once to twice per day in the morning and late afternoon, as needed. Neptune's Fish fertilizer (Neptune's Harvest, Gloucester, ME) was applied to all seedlings 2-3 times 7-14 d after sowing. Supplemental pest and disease management was not applied as the seedlings appeared to be healthy.

Pre-grafting light treatment

Pre-grafting light treatment began on 20 Oct. 2013, 20 Nov. 2013, and 9 July 2014 in Repeats 1, 2, and 3, respectively, and lasted 5 d until seedlings were grafted. One set of 'Celebrity' and 'Maxifort' seedlings was placed in a growth chamber (Conviron Model; Controlled Environments Ltd., Manitoba, Canada) providing 250 µmol/m^2/s for 14 h (0600-2000 $_{HR}$) from 400-W metal halide lamps (Multi-Vapor®; GE Lighting, East Cleveland, OH) and 400-W high-pressure sodium lamps (Ultra Sun®; Sunlight Supply, Vancouver, WA). A second, equal-sized set of 'Celebrity' and 'Maxifort' seedlings was placed in an adjacent, identical growth chamber with no illumination (24-h dark). Temperature and relative humidity set points in both growth chambers were 24 °C (0600-2000 $_{HR}$), 20 °C (2000-0600 $_{HR}$), and 40%, respectively. Overhead irrigation was applied manually twice daily.

Grafting

All 'Maxifort' and a subset of 'Celebrity' seedlings were grafted at the conclusion of the 5-d pre-grafting light treatment. All grafts were completed on the same day by one experienced grafter using the cleft method (Bumgarner and Kleinhenz, 2013). Self-grafted 'Celebrity' control plants were made using portions of the same plant while standard grafted plants included 'Maxifort' and 'Celebrity' as rootstock and scion, respectively.

Post-grafting light treatment

Post-grafting light treatment was carried out using the same chambers and temperature set points described above. However, humidity was set at 95% in both chambers and the light intensity in one chamber was 135 µmol/m^2/s (0600-2000 $_{HR}$). One set of all types of plants (grafted, self-grafted, ungrafted) was placed in the lighted chamber while a second, equal-sized

set of all types of plants was placed in an adjacent, identical chamber with no illumination (24-h dark). Twenty-four to forty plants of each treatment were placed in the growth chamber.

Three types of grafting (grafted, self-grafted, ungrafted) and two levels of pre- and post-grafting light treatment (dark, light) create the potential for a total of twenty grafting-light treatment and control groups (Table 6). Ten of those groups were included in this study (Table 6). Note that self- and standard grafted plants were made from seedlings exposed to the same pre-grafting light treatment. Other groups in Table 6 represent intriguing possibilities for follow-up work, especially when the influence of energy reserves and production on early-stage grafted-plant growth is of interest.

Graft healing measurement

Six to ten plants from each treatment and control group were measured 3, 5, 7 and 9 d after grafting. Survival rate was calculated using the numbers of grafted plants with a wilted or healthy scion (Johnson and Miles 2011). Plants with a completely wilted scion were scored as dead; others as living. Stem diameter was measured at the midpoint of the rootstock of grafted and self-grafted plants or the hypocotyl of ungrafted plants with a digital caliper (Traceable®; Control Company, Friendswood, TX). Plant height from the soil line to the meristem and scion length from the graft union to the meristem were measured with a ruler. Plants were then excised at the soil line and placed into a solution containing 7 mg/ml Erythrosin B dissolved in distilled deionized water. After 15 min, the stain terminus was identified by carefully removing the epidermis of the scion stem ('epidermal peel') with a hand-held blade (Rock River Single Edge Blades; distributed by Fastenal Company, Winona, MN) and locating, without magnification, the point at which dye was no longer evident. The distance of dye movement was measured from where the plant was cut to the stain terminus (stained length), and from the graft union to the stain terminus (stained length above the graft union). The percentage of plants with stain above the graft union was calculated as the number of plants with stain above the graft union/the number of plants measured×100%.

Data analysis

A factorial design was used to study the effects of two levels of pre-grafting and two levels of post-grafting light treatments on grafted plants. Self- and ungrafted controls under different light treatments were also included. Growth was recorded on day 9, while survivorship and dye movement were measured on days 3, 5, 7 and 9.

To test the separate and interactive effects of pre- and post-grafting light treatment, analysis of variance using data from grafted plants was conducted with pre-grafting, post-grafting, pre-/post-grafting interaction and repeat as fixed factors in the GLIMMIX procedure in SAS (version 9.3; SAS Institute, Cary, NC). To compare grafted plants to self-grafted and ungrafted controls under different light treatments, analysis of variance among the ten grafting-light treatments was conducted with treatment and repeat as fixed factors using the GLIMMIX procedure. Means separation was completed using the LINES and BYLEVEL options in the LSMEANS statement in the GLIMMIX procedure (alpha = 0.05).

Results

Self-grafting differentiates effects due to wounding (the grafting process only) from ones resulting from the additive influences of grafting and the use of different rootstock and scion genetics. In this experiment, self- and standard grafted plants exhibited similar responses to their respective light treatments through 9 d after grafting, although standard grafted plants had higher levels of survival (days 7 and 9), plant height and scion length (day 9), as well as dye movement (days 5, 7 and 9) than self-grafted plants under the same light treatment. This result may reflect the greater vigor of ‘Maxifort’ than ‘Celebrity’ root systems. However, the overall similarity between self- and standard grafted data clear the way to focus on light treatment effects on standard grafted seedlings versus ungrafted ones.

Pre- and post-grafting light treatment and their interactions had significant effects on multiple variables in grafted plants (Tables 7-9). Pre-grafting light treatment affected survivorship

(on days 7 and 9), stem diameter (on day 9) and dye movement (on days 7 and 9). Post-grafting light treatment affected survivorship (on days 7 and 9), scion length (on day 9) and dye movement (on days 5, 7 and 9). The interactions of pre- and post-grafting light treatment were significant in survivorship (on days 7 and 9) and dye movement (on days 7 and 9).

Survivorship of grafted plants did not differ among light treatments, except for that under continual darkness pre- and post-grafting, where survival was lower and reduced over time (Table 10). Continual darkness also resulted in plant death in ungrafted plants on days 7 and 9.

Growth differed among plants exposed to the four light treatments (Table 11), in patterns specific to the grafting group (grafted and ungrafted). Among ungrafted plants, stem diameter was greater in plants exposed to light than dark. Similarly, among standard grafted plants, stem diameter was greater in plants exposed to light than dark before grafting, regardless of light or dark conditions after grafting. Ungrafted plants had larger plant height in dark versus light. In contrast, plant height and scion length of grafted plants were greater in plants exposed to light versus dark after grafting, regardless of whether seedlings used to prepare grafted plants were exposed to light or dark for 5 d before grafting.

Three trends were evident in the data collected by tracking the movement of dye through the graft union (Tables 12-13). First, dye movement in grafted plants increased from 3 to 9 d after grafting, achieving values comparable to those of ungrafted control plants within 5 to 7 d after grafting, provided grafted plants were given light after grafting. Second, dye movement was greater in standard- than self-grafted plants under the same light treatment. Third, providing standard- or self-grafted plants with light after grafting promoted dye movement, regardless of whether seedlings used to prepare grafted plants were exposed to light or dark for 5 d before grafting.

The percentages of plants with stain above the graft union or cotyledons were lower in grafted plants than ungrafted controls under the same light treatment (Table 12). Grafted plants achieved comparable percentages as their ungrafted counterparts since 7 d after grafting under

Dark/Light, and since 5 d after grafting under Light/Light. In grafted plants, the percentages were higher under light versus dark after grafting on days 5, 7 and 9, regardless of the light condition before grafting.

The percentages of stained length to plant height were lower in grafted plants than ungrafted ones under the same light treatment (Table 13). The dye movement in ungrafted plants almost always reached the meristem (close to 100% of stained length to plant height), with no difference among the four light treatments. Grafted plants achieved comparable percentages as their ungrafted counterparts 5, 7 and 9 d after grafting under Dark/Light, and 7 d after grafting under Light/Light. In grafted plants, the percentages were higher under light versus dark after grafting on days 5, 7 and 9, regardless of the light condition before grafting.

Consistent trends were observed in stained length above the graft union and its percentage to scion length (Figures 2-3). Dye movement through the graft union increased over time after grafting. In grafted plants, the values of these two variables were larger under light versus dark after grafting on days 5, 7 and 9.

Discussion

Graft survivorship did not differentiate grafted tomato seedlings under different light treatments pre- and post-grafting, except for the continual dark condition both pre- and post-grafting. The high survivorship (above 80%) of grafted tomato plants in the other three light treatments was consistent with a previous study reported by Johnson and Miles (2011). In the continual dark treatment, the low survivorship of grafted plants might be due to the continuous inhibition of photosynthesis and failure of graft healing. Similar results were found by Vu et al. (2014a) with 27.8% survival when tomato seedlings were grown in darkness for 10 d before grafting, and 68.5% survival in darkness for 10 d after grafting.

Plant growth variables were able to differentiate groups of seedlings under different light treatments pre- and post-grafting. Light regulates morphological development in seedlings

through photomorphogenesis and skotomorphogenesis (Josse and Halliday, 2008; Von Arnim and Deng, 1996). Seedlings grown in dark developed long hypocotyls (Hou et al., 2002), which was consistent with the results of this study that light exposure resulted in shorter plant height in ungrafted plants. However, in grafted plants, plant height and scion length were larger under light versus dark post-grafting. The different trends of plant elongation under different light treatments between grafted and ungrafted plants might be caused by the interactive effects of light exposure on plant growth and on graft healing. Grafting creates a physical wound and causes suspension of plant growth. Light exposure after grafting stimulated healing and growth resumption of grafted plants (the positive effect of light), even though light inhibited plant stem elongation (the negative effect of light). The positive effect of light on healing was larger than the negative effect on growth, so larger plant height and scion length in grafted plants were achieved under the post-grafting light condition.

Plant growth parameters especially stem elongation, seemed to be a better healing indicator than the survivorship for grafted plants under different light treatments. However, morphological indicators alone may be not sufficient or reliable for graft healing assessment due to the confounding effects of light on plant growth.

Dye movement differentiated groups of plants under different light treatments. In ungrafted plants, water flow exists throughout the plant, and the dye can transport upward to the meristem. In grafted plants before they heal, the graft interface becomes a barrier for water transportation from the rootstock to the scion. Graft healing involves cell division and then wound vessel differentiation resulting in the vascular interconnection between the rootstock and the scion (Pina and Errea, 2005; Trinchera et al., 2013). Vascular reconnection enabling water and nutrient exchange through the graft union is generally regarded as an indicator of graft healing (Martínez-Ballesta et al., 2010; Wang and Kollmann, 1996). A translocatable dye allows visualization of the graft healing process and is used to confirm vascular tissue re-formation. Immediately after grafting, water transportation by bulk flow between the rootstock and the scion

is terminated. The dye solution absorbed from the rootstock accumulates at the graft union, but can not pass through the barrier after an observation period of 15 min. After the graft union heals and a functioning and continuous vascular system from the rootstock to the scion is formed, the dye is transported within the xylem through the action of bulk flow. The dye movement observed here is considered to be due to bulk flow rather than diffusion of the dye solution. Even though diffusion probably occurs simultaneously, it would be much slower than the bulk flow. Therefore, the stain observed 15 min after placing the plants in the dye solution would be primarily from the long-distance transport of the bulk flow, reflecting vascular functioning.

The percentage of plants with stain above the graft union and the percentage of stained length to plant height in grafted and self-grafted plants increased over time from 3 to 7 d after grafting (except for the continual dark treatment), and remained stable on day 9. This result suggested that graft union developed gradually until it reached complete healing. Under the light condition post-grafting, grafted plants reached comparable percentages as ungrafted controls after 7 d of grafting. Therefore, it took 7 d for grafted plants to heal and resume vascular function comparable to their ungrafted counterparts under the healing conditions applied in this study with light exposure post-grafting. The time required for healing of grafted tomato plants is not identical compared to previous studies since healing conditions are different among these studies, but the range of time is consistent. Fernández-García et al. (2004) found that vascular connection through the graft union formed and fully functioned 8 d after grafting in tomato. Turquois and Malone (1996) concluded that hydraulic reconnection between tomato rootstocks and scions appeared over 48 h from 5 d after grafting. Lee et al. (2016) reported that vascular connection and functioning resumed 50% in the first 5 d.

The method developed in this study, especially using stem elongation to monitor plant regrowth and Erythrosin B to visualize rootstock-scion reconnection, successfully quantified the gradual graft healing process. Plant survival and growth measures taken on one day are useful assessments of grafted-plant condition; dye movement monitored on multiple days represents an

important, additional opportunity to monitor healing over time. The plant growth and dye measurement has a higher resolution than the survival rating, and it is fast, easy to use without the requirement of specific equipment or techniques. The dye movement observed in this approach can differentiate variable healing responses as early as 5 d after grafting. One possible limitation of this dye approach may be the confounding effects from transpiration and diffusion. Under different treatments, plants may result in different transpiration rates, varying the pressure gradient and bulk flow, which may influence the distance of dye transport. Therefore, the possible effects of transpiration should be considered when interpreting dye transport results. In this study, plants were placed under identical conditions during the dye assay and the influence of transpiration should be minimal. Diffusion of the dye can be ignored since diffusion is much slower than bulk flow and dye movement was observed 15 min after exposing the plants to the dye solution. Other previous attempts have been made for recording the graft union re-formation, such as histological observation at the cellular level, water potential and xylem hydraulic conductivities (Kawaguchi et al., 2008), and tensile strength measurement (Moore, 1983; Roberts and Brown, 1961). A most recent report demonstrated methods to monitor vascular functioning by measuring transpiration, water uptake rate, sugar content and flower dye distributions (Lee et al., 2016). Other studies also applied tracers and dyes to visualize vascular systems as well as water and nutrient flow from the rootstock to the scion, including radioactive tracers such as ^{14}C-sucrose (Wang and Kollmann, 1996) and fluorescence staining (Melnyk et al., 2015; Yin et al., 2012), acid fuchsin (Robb et al., 1983; Yin et al., 2012), Safranin O (Aloni et al., 2008), methyl blue (Johkan et al., 2009; Oda et al., 2005), and flower staining dye (Lee et al., 2016). Erythrosin B was used in this study for visualizing vascular functioning, showed a bright red color, and was effective to indicate water flow in grafted plants without the requirement of specific equipment for observation.

Light conditions both pre- and post-grafting affected plant growth and dye movement in grafted and self-grafted plants. The post-grafting light treatments, especially, seemed to play a

more important role in graft healing. Plant height, scion length and dye movement were larger under light versus dark post-grafting, regardless of the condition before grafting. The results may suggest that the post-grafting light exposure stimulated graft healing and thus increasing plant regrowth and dye transportation from the rootstock to the scion. The results from this study were consistent with previous studies which found enhanced graft success and growth of grafted tomatoes under light versus dark post-grafting (Vu et al., 2014b), and no significant difference in early growth of grafted cucumber under high and low light intensities pre-grafting (Kwack et al., 2014). Higher light intensity at 100 μmol/m^2/s during healing increased protein expression related to vascular connection, and increased biomass and graft union hardness in grafted watermelon on bottle gourd (Muneer et al., 2015). These results may suggest that energy assimilation and/or light regulation after grafting plays an important role in the healing process, which needs to be further studied.

The ratio of stained length to plant height was affected by the original plant size and plant growth, and thus might lead to confounding results. For example, grafted plants under Dark/Dark had a numerically or significantly higher percentage of stained length to plant height than those from the other three light treatments 3 d after grafting. This, however, was not necessarily equal to further dye movement above the graft union under Dark/Dark, because plants grown in dark developed a longer hypocotyl and after they were grafted, a higher rootstock length, resulting in a higher percentage of stained length to plant height even without dye movement above the graft union. To clarify the confounding effects, the actual stained length above the graft union and its percentage to the scion length were also compared (Figures 2-3). The same trend was shown that grafted and self-grafted plants under light versus dark after grafting had dye traveled to a longer distance above the graft union.

The self-grafted control had less dye movement than grafted plants. The difference between self-grafted and grafted plants was the rootstock portion, which might be responsible for this different trends. In grafted plants, the rootstock was ‘Maxifort’, a rootstock cultivar bred to

have a strong root system; while in self-grafted plants, the rootstock was ‘Celebrity’, a scion cultivar which probably has a poorer root system than ‘Maxifort’. The weaker roots in self-grafted plants might cause the lower graft healing rate compared to that of grafted plants. Weng (2000) found consistent results that grafted tomato plants had a greater ability of water uptake than self-grafted tomato plants.

To conclude, survivorship was not able to differentiate groups of grafted seedlings under various light treatments except for the continual darkness by 9 d after grafting. Morphological assessment of plant growth (stem elongation) was able to track different healing responses to light treatments. Dye movement in grafted plants is an indirect visual indicator of the gradual process of vascular reconnection and functioning, and differentiated healing as early as 5 d after grafting. The data of plant growth measurement and dye movement reinforced each other, suggesting that light exposure post-grafting might promote healing of grafted tomato seedlings.

Table 6. Pre- and post-grafting light and grafting treatments listed in the columns 1-4 (left-right) below create a total of twenty possible treatments. Ten of those possible treatments resulting from the use of both pre- and post-grafting light treatments, all three grafting treatments, and the possible combinations of seedling stem and root portions marked with an asterisk combined with both light and dark treatment after grafting were included in this study

Light intensity (µmol/m^2/s) treatment of seedlings for 5 d immediately prior to grafting	Grafting treatment	Possible combinations of seedling stem and root portions at grafting	Light intensity (µmol/m^2/s) treatment for 9 d immediately after Grafting	Total no. of possible treatments (number used in study)
250 (light) 0 (dark)	Standard Grafted 'Maxifort' /'Celebrity'	'Max' Light/'Cel' Light* 'Max' Light/'Cel' Dark 'Max' Dark/'Cel' Light 'Max' Dark/'Cel' Dark*	135 (light) 0 (dark)	8 (4)
	Self-grafted 'Celebrity' /'Celebrity'	Light/Light (same plant)* Dark/Dark (same plant) Light/Dark (different plants) Dark/Light (different plants)		8 (2)
	Ungrafted 'Celebrity'	Light* Dark*		4 (4)

Table 7. *P* values of type III tests for main and interactive effects of pre- and post-grafting light treatments on survivorship of grafted tomato seedlings 3, 5, 7 and 9 d after grafting

Source of variance[z]	Days after grafting			
	3	5	7	9
Pre-grafting	0.07	0.08	0.0001	<.0001
Post-grafting	0.2	0.4	0.0002	<.0001
Interaction	0.2	0.4	0.0003	<.0001

[z] Pre-grafting light treatments include placing seedlings in environmentally controlled chambers maintained at 0 or 250 µmol/m^2/s for 5 d immediately before grafting; post-grafting light treatments include placing grafted plants in chambers maintained at 0 or 135 µmol/m^2/s for 9 d immediately after grafting.

Table 8. *P* values of type III tests for main and interactive effects of pre- and post-grafting light treatments on plant growth of grafted tomato seedlings 9 d after grafting

Source of variance[z]	Stem diam[y]	Plant ht[x]	Scion length[w]
Pre-grafting	0.009	0.5	0.2
Post-grafting	0.6	0.07	0.03
Interaction	0.6	0.5	0.8

[z] Pre-grafting light treatments include placing seedlings in environmentally controlled chambers maintained at 0 or 250 µmol/m^2/s for 5 d immediately before grafting; post-grafting light treatments include placing grafted plants in chambers maintained at 0 or 135 µmol/m^2/s for 9 d immediately after grafting.
[y] Stem diameter was measured at the middle of the rootstock of grafted plants.
[x] Plant height was measured from the soil line to the meristem.
[w] Scion length was measured from the graft union to the meristem.

Table 9. *P* values of type III tests for main and interactive effects of pre- and post-grafting light treatments on dye movement of grafted tomato seedlings 3, 5, 7 and 9 d after grafting

Source of variance[z]	Percentage of plants with stain above the graft union[y]				Percentage of stained length to plant ht[y]			
	3	5	7	9	3	5	7	9
Pre-grafting	0.6	0.2	0.01	0.01	0.1	0.5	0.02	0.7
Post-grafting	0.2	0.02	0.001	0.0001	0.7	<.0001	<.0001	0.06
Interaction	0.2	0.6	0.1	0.05	0.1	0.7	0.02	0.4

[z] Pre-grafting light treatments include placing seedlings in environmentally controlled chambers maintained at 0 or 250 µmol/m^2/s for 5 d immediately before grafting; post-grafting light treatments include placing grafted plants in chambers maintained at 0 or 135 µmol/m^2/s for 9 d immediately after grafting.

[y] Plants were excised at the soil line and placed in a 7 mg/ml Erythrosin B solution for 15 min, and then the dye movement was observed. The percentage of plants with stain above the graft union was calculated as the number of plants with stain above the graft union/the number of plants measured×100%. The stained length from the soil line to the stain terminus was measured. The percentage of stained length to plant height (from the soil line to the meristem) was calculated.

Table 10. Survivorship 3, 5, 7 and 9 d after grafting of grafted tomato seedlings and self-grafted and ungrafted controls under four pre- and post-grafting light treatments

Plants	Conditions pre/post grafting[z]	Days after grafting			
		3	5	7	9
Grafted	Dark/Dark	56 b[y]	56 b	22 c	6 d
	Dark/Light	89 a	83 ab	94 a	100 a
	Light/Dark	97 a	100 a	97 a	100 a
	Light/Light	97 a	100 a	100 a	100 a
Self-grafted	Light/Dark	94 a	94 a	67 b	67 bc
	Light/Light	94 a	100 a	100 a	94 ab
Ungrafted	Dark/Dark	100 a	100 a	83 ab	39 c
	Dark/Light	100 a	100 a	100 a	100 a
	Light/Dark	100 a	100 a	100 a	100 a
	Light/Light	100 a	100 a	100 a	100 a

[z] Pre-grafting light treatments include placing seedlings in environmentally controlled chambers maintained at 0 (dark) or 250 (light) µmol/m^2/s for 5 d immediately before grafting; post-grafting light treatments include placing grafted plants and controls in chambers maintained at 0 (dark) or 135 (light) µmol/m^2/s for 9 d immediately after grafting.

[y] Each data point is the mean of three repeats. Means within a column followed by the same letter are not significantly different ($P < 0.05$) analyzed using the LINES and BYLEVEL options in the LSMEANS statement in the GLIMMIX procedure.

Table 11. Stem diameter, plant height, and scion length 9 d after grafting of grafted tomato seedlings and self-grafted and ungrafted controls under four pre- and post-grafting light treatments

Plants	Conditions pre/post-grafting[z]	Stem diam (mm)[y]		Plant ht (cm)[x]		Scion length (cm)[w]	
Grafted	Dark/Dark	1.58	NA[v]	3.50	NA	1.50	NA
	Dark/Light	1.42	c	4.04	cd	1.48	ab
	Light/Dark	1.65	b	3.31	ef	1.29	bc
	Light/Light	1.65	b	3.67	de	1.68	a
Self-grafted	Light/Dark	1.73	ab	2.78	g	1.01	d
	Light/Light	1.77	a	3.05	fg	1.21	cd
Ungrafted	Dark/Dark	1.47	c	4.76	b	NA	
	Dark/Light	1.70	ab	5.54	a	NA	
	Light/Dark	1.74	ab	5.49	a	NA	
	Light/Light	1.79	a	4.16	bc	NA	

[z] Pre-grafting light treatments include placing seedlings in environmentally controlled chambers maintained at 0 (dark) or 250 (light) µmol/m^2/s for 5 d immediately before grafting; post-grafting light treatments include placing grafted plants and controls in chambers maintained at 0 (dark) or 135 (light) µmol/m^2/s for 9 d immediately after grafting.

[y] Stem diameter was measured at the middle of the rootstock of grafted and self-grafted plants and at the middle of the hypocotyl of ungrafted controls. 1 mm = 0.0394 inch.

[x] Plant height was measured from the soil line to the meristem. 1 cm = 0.3937 inch.

[w] Scion length was measured from the graft union to the meristem. Not available in ungrafted plants. 1 cm = 0.3937 inch.

[v] Each data point is the mean of three repeats, except for the treatment 'Grafted Dark/Dark' which had only one plant living in repeat three, and its data is not included for multiple comparisons among treatments. Means within a column followed by the same letter are not significantly different ($P < 0.05$) analyzed using the LINES and BYLEVEL options in the LSMEANS statement in the GLIMMIX procedure.

Table 12. Percentage of plants with stain above the graft union (grafted and self-grafted tomato seedlings) or the cotyledons (ungrafted controls) under four pre- and post-grafting light treatments 3, 5, 7 and 9 d after grafting

Plants	Conditions pre/post grafting[z]	Days after grafting							
		3		5		7		9	
Grafted	Dark/Dark	5.6[y]	bc	11.1	e	0.0	d	0.0	NA[x]
	Dark/Light	5.6	bc	61.1	bc	83.3	ab	77.8	ab
	Light/Dark	0.0	c	40.0	cd	53.3	c	46.7	c
	Light/Light	20.0	b	76.7	ab	96.7	a	90.0	a
Self-grafted	Light/Dark	0.0	c	4.2	e	20.8	d	13.9	d
	Light/Light	19.4	b	30.6	de	70.8	bc	63.9	bc
Ungrafted	Dark/Dark	100.0	a	88.9	ab	100.0	a	100.0	a
	Dark/Light	100.0	a	100.0	a	94.4	ab	100.0	a
	Light/Dark	94.4	a	94.4	a	100.0	a	94.4	a
	Light/Light	100.0	a	94.4	a	100.0	a	100.0	a

[z] Pre-grafting light treatments include placing seedlings in environmentally controlled chambers maintained at 0 (dark) or 250 (light) µmol/m^2/s for 5 d immediately before grafting; post-grafting light treatments include placing grafted plants and controls in chambers maintained at 0 (dark) or 135 (light) µmol/m^2/s for 9 d immediately after grafting.

[y] Plants were excised at the soil line and placed in a 7 mg/ml Erythrosin B solution for 15 min, and then the dye movement was observed. The percentage of plants with stain above the graft union was calculated as the number of plants with stain above the graft union/the number of plants measured×100%.

[x] Each data point is the mean of three repeats, except for the treatment 'Grafted Dark/Dark' on day 9 which had only one plant living in repeat three, and its data is not included for multiple comparisons among treatments. Means within a column followed by the same letter are not significantly different ($P < 0.05$) analyzed using the LINES and BYLEVEL options in the LSMEANS statement in the GLIMMIX procedure.

Table 13. Percentage of stained length to plant height of grafted tomato seedlings and self-grafted and ungrafted controls under four pre- and post-grafting light treatments 3, 5, 7 and 9 d after grafting

Plants	Conditions pre/post-grafting[z]	Days after grafting							
		3		5		7		9	
Grafted	Dark/Dark	66.5[y]	b	62.8	c	52.2	d	57.1	NA[x]
	Dark/Light	59.9	bc	86.4	ab	92.4	ab	92.7	ab
	Light/Dark	55.7	c	69.3	c	77.9	c	77.6	cd
	Light/Light	59.7	bc	86.3	b	92.3	ab	88.7	b
Self-grafted	Light/Dark	55.5	c	58.9	c	61.1	d	69.3	d
	Light/Light	58.6	bc	69.8	c	86.7	bc	85.1	bc
Ungrafted	Dark/Dark	91.2	a	91.9	ab	93.7	ab	97.3	ab
	Dark/Light	96.2	a	97.6	a	95.3	ab	100.0	a
	Light/Dark	95.1	a	95.6	ab	98.7	a	97.1	ab
	Light/Light	95.7	a	97.2	a	94.5	ab	99.3	a

[z] Pre-grafting light treatments include placing seedlings in environmentally controlled chambers maintained at 0 (dark) or 250 (light) µmol/m^2/s for 5 d immediately before grafting; post-grafting light treatments include placing grafted plants and controls in chambers maintained at 0 (dark) or 135 (light) µmol/m^2/s for 9 d immediately after grafting.

[y] Plants were excised at the soil line and placed in a 7 mg/ml Erythrosin B solution for 15 min, and the stained length from the soil line to the stain terminus was measured. The percentage of stained length to plant height (from the soil line to the meristem) was calculated.

[x] Each data point is the mean of three repeats, except for the treatment 'Grafted Dark/Dark' on day 9 which had only one plant living in repeat three, and its data is not included for multiple comparisons among treatments. Means within a column followed by the same letter are not significantly different ($P < 0.05$) analyzed using the LINES and BYLEVEL options in the LSMEANS statement in the GLIMMIX procedure.

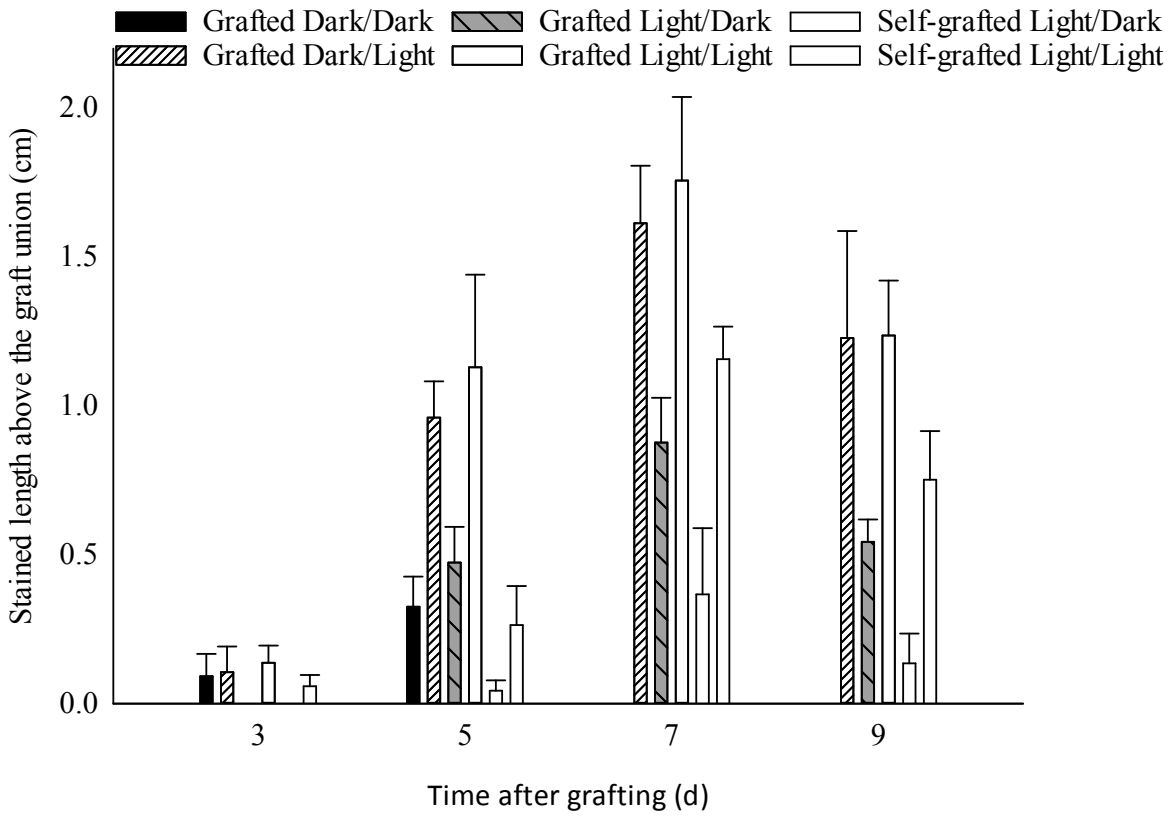

Figure 2. Stained length above the graft union of grafted tomato seedlings and self-grafted controls under four pre- and post-grafting light treatments 3, 5, 7 and 9 d after grafting. Pre-grafting light treatments include placing seedlings in environmentally controlled chambers maintained at 0 (dark) or 250 (light) µmol/m^2/s for 5 d immediately before grafting; post-grafting light treatments include placing grafted plants and controls in chambers maintained at 0 (dark) or 135 (light) µmol/m^2/s for 9 d immediately after grafting. Plants were excised at the soil line and placed in a 7 mg/ml Erythrosin B solution for 15 min and the stained length above the graft union was measured from the graft union to the stain terminus. 1 cm = 0.3937 inch. Each data point is the mean of three repeats, except for the treatment Grafted Dark/Dark on day 9, which had only one plant living in repeat three. Error bars are standard errors. Means on day 5, 7, and 9 among the six treatments were significantly different ($P < 0.05$) analyzed using the LINES and BYLEVEL options in the LSMEANS statement in the GLIMMIX procedure

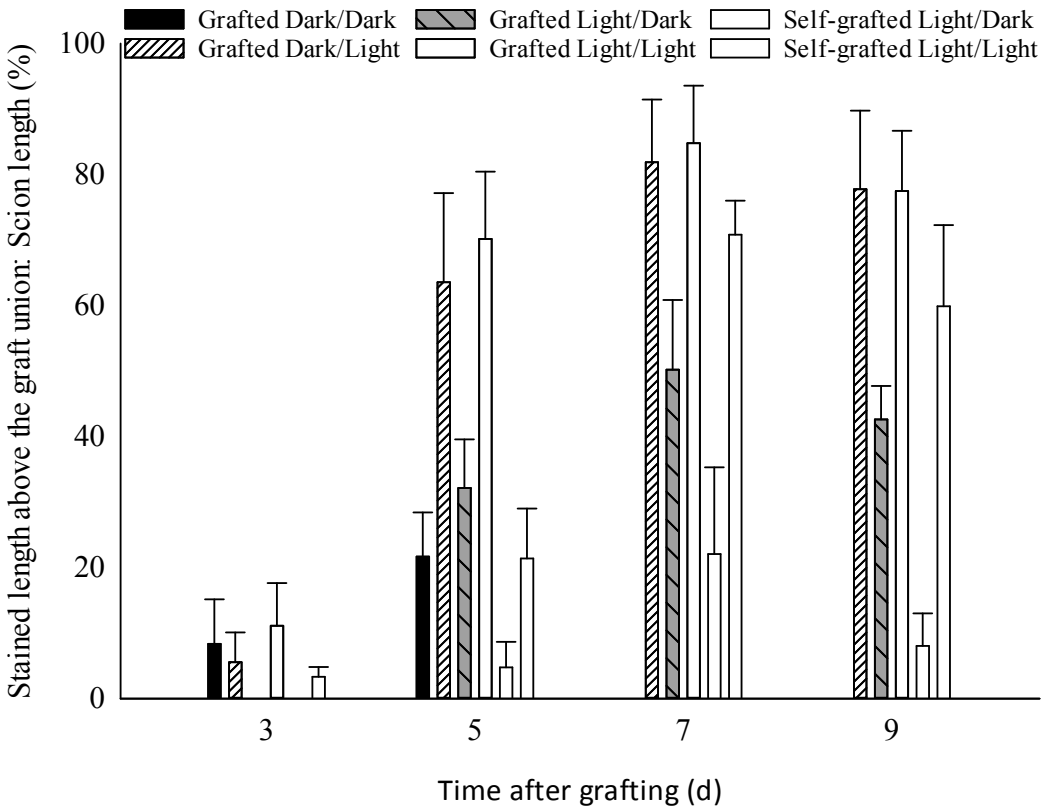

Figure 3. Percentage of stained length above the graft union to the scion length of grafted tomato seedlings and self-grafted controls under four pre- and post-grafting light treatments 3, 5, 7 and 9 d after grafting. Pre-grafting light treatments include placing seedlings in environmentally controlled chambers maintained at 0 (dark) or 250 (light) µmol/m^2/s for 5 d immediately before grafting; post-grafting light treatments include placing grafted plants and controls in chambers maintained at 0 (dark) or 135 (light) µmol/m^2/s for 9 d immediately after grafting. Plants were excised at the soil line and placed in a 7 mg/ml Erythrosin B solution for 15 min and the stained length above the graft union was measured from the graft union to the stain terminus. Scion length was measured from the graft union to the meristem. Each data point is the mean of three repeats, except for the treatment Grafted Dark/Dark on day 9, which had only one plant living in repeat three. Error bars are standard errors. Means on day 5, 7, and 9 among the six treatments were significantly different ($P < 0.05$) analyzed using the LINES and BYLEVEL options in the LSMEANS statement in the GLIMMIX procedure

Chapter 4: Light Intensity during the Healing Period Affects Plant Regrowth of Grafted Tomato Seedlings

Introduction

Light is one of the most important environmental factors regulating plant growth and development. Adjustments in light quality, intensity, and duration have gained practical application to plant cultivation and food production (Chia and Kubota, 2010; Ehret et al., 1989). In grafted-vegetable propagation, light management during the healing process is important to graft success and high quality of grafted seedlings (Lee et al., 2016; Nguyen et al., 2014; Vu et al., 2014a).

Healing of grafted seedlings is an energy demanding process (Tornbom and Oliveira, 1993). It includes complex physiological interactions between rootstocks and scions (Martínez-Ballesta et al., 2010). Light is the ultimate source to meet this energy requirement through photosynthetic energy conversion.

In addition, graft healing involves a series of biochemical signaling pathways (Howe, 2004; Yin et al., 2012). Rootstock-scion communication is regulated by multiple phytohormones. Light may regulate plant development, differentiation and growth during the healing process through photoreceptors and phytohormones.

Even though light is likely to influence graft healing as discussed above, it is unclear what is the optimal light intensity during the healing of grafted tomato (*Solanum lycopersicum*) seedlings. Only a limited number of studies without a consistent conclusion are available on the light management for the propagation of grafted tomato seedlings. Vu et al. (2014b) reported that

15 μmol/m^2/s from red LED achieved higher survival and plant growth in grafted tomato seedlings compared to darkness during the healing period. Nobuoka et al. (1996) recommended lower light intensity among 0, 77 and 465 μmol/m^2/s, while Nobuoka et al. (2005) suggested higher light intensity at 114 μmol/m^2/s compared to 57 μmol/m^2/s for efficient healing of grafted tomato plants. Based on these two report, the optimal light intensity for grafted tomato healing may be between 114 and 465 μmol/m^2/s. Peat (1970) determined that the photosynthesis saturation point for young, ungrafted tomato seedlings was about 150 W/m^2 from tungsten bulbs. The saturation point of grafted tomato seedlings may be different from 150 W/m^2 due to the physical wound and the reduced leaf area with leaves trimmed at grafting (Masterson et al., 2016). Since the newly grafted plant has limited photosynthetic capacity but is under an energy-demanding healing process, adequate light may be beneficial for rapid graft union formation (Lee and Oda, 2010), but excessive light might be detrimental. Too much light maintained in healing chambers can cause a waste of energy, an unfavorable increase in temperature and plant transpiration, and harm to the grafted plants (Barber and Andersson, 1992).

The objective of this study was to test the effects of light intensity on plant regrowth of grafted tomato seedlings. The study was conducted in a controlled environment with uniform air temperature and humidity among treatments, so as to examine the effects of light intensity without confounding interactions with temperature and humidity which may also affect graft healing. Light emitting diode (LED) was used as the sole light source to more reliably create various light intensities while minimizing the effects on the temperature around plant tissues due to the cool emitting surface of LED. Results from this study can provide guidance for optimal light management to enhance the efficiency of grafted tomato propagation.

Materials and Methods

The studies were conducted at the Ohio Agricultural Research and Development Center in Wooster, OH. Two studies with the same hypothesis and light intensity treatments were carried

out in two different healing chambers. Study one was conducted twice from 13 Apr. 2015 to 1 June 2015 in a highly environmental-controlled plant growth chamber; study two was conducted twice from 15 May 2015 to 25 June 2015 in a low environmental-controlled healing chamber constructed in the lab.Tomato rootstock 'Maxifort' from Johnny's Selected Seeds (Winslow, ME) and tomato scion 'Cherokee Purple' from NE Seed (Harford, CT) were used for both studies.

Plant materials

Seedlings were grown in an environmentally controlled greenhouse with the temperature set as 22-26 °C from dawn to dusk, and 17-21 °C from dusk to dawn. HID lights came on at dawn and stayed on for 16 h unless outdoor sunlight stayed above 350 W/m^2 for 20 minutes. Seed was sown in 96-cell plug trays preloaded with growing medium (Pro-Mix® MP Mycorrhizae™ Organik™; Premier Tech, Quebec, Canada). Trays were placed on one layer of a capillary mat (Kapmat; Buffalo Felt Products Corp, West Seneca, NY) underlain by one layer of clear plastic on a 5.4 m x 1.8 m bench in the greenhouse. Trays were hand-misted to wetness immediately after sowing; an automated irrigation system was used thereafter for 1 week to maintain medium moisture, with 1 fogger (each delivered a flow rate of 8.1 gal/h) over each tray pulsed on for 8 s every 30 min and 2 drippers (each with a flow rate of 1.5 gal/h) on the capillary mat underneath each tray pulsed on for 3 min at 0700, 1100 and 1500 $_{HR}$ every day. One week after seed sowing, seedlings were placed on a heat mat controlled by a thermostat (Redi Heat; Phytotronics, Inc. Earth City, MO) at 29 °C on a 5.4 m x 1.25 m bench without automatic irrigation systems, and hand-misted overhead once to twice a day depending on the weather.

Each seedling was marked by the date of their emergence. One day before grafting, seedlings with similar sizes and emergence dates were sorted for rootstocks and scions, and assigned to one block to reduce plant variations within a block and ensure matching in the stem diameter between rootstocks and scions.

Grafting procedure

Three weeks after seeding, 'Cherokee Purple' seedlings were grafted onto 'Maxifort' using the splice grafting method as outlined previously (Hu et al., 2016b) by the same experienced grafter. 'Maxifort' seedlings were decapitated right below the cotyledons at a 45° angle. 'Cherokee Purple' seedlings were derooted where stem diameter matched with that at the rootstock cut surface. Then the two plant tissues were put together and secured by a spring grafting clip. The old leaves were trimmed, leaving a consistent amount of new leaves on the scion (Figure 4A).

Light intensity treatments post-grafting

Both studies used the same LED light (WEX-C150; Welthink Electronic America, Virgina Beach, VA) containing 60% blue (460 nm), 20% red (630 nm) and 20% white as the sole light source with a 12 h photoperiod during the healing period after grafting. Four light intensities (5, 50, 150, 300 μmol/m^2/s) were created by differing the distances between plants and the light source and using a black knitted shade cloth (50% PAR transmission; Tek Inc., Janesville, WI), as the four light intensity treatments under each LED fixture in each block. Both studies were performed as a randomized complete block design, with four blocks in study one and three blocks in study two.

Study one

Immediately after grafting, plants were placed in a plant growth chamber (Thermolinear, Cincinnati, OH) for 10 d. The temperature was set at 25 (0600-1800 $_{HR}$)/20 °C (1800-0600 $_{HR}$) in the growth chamber during the study period. Relative humidity in the chamber was set at 80% in the first 7 d after grafting, then 70% on the 8th day, and 60% on the 9th and 10th day. Four LED fixtures were installed in the growth chamber representing four blocks.

Ten plants for each treatment each block were placed on two layers of capillary mat pre-wetted by 500 ml water, underneath by one layer of clear plastic sheeting. The rooting medium and capillary mat were watered manually when they appeared dry during the 10 d.

Ten days after grafting, half of the plants from each treatment were destructively measured as described below. The other half were moved to the same environmentally controlled greenhouse with the same temperature and light conditions as for seeding for 7 d. Plants were put on one layer of capillary mat underlain by one layer of clear plastic on a 5.4 m x 1.8 m bench. The capillary mat was manually misted wet before plants were put on. Thereafter, medium moisture was maintained using an automatic irrigation system with four drippers (each with a flow rate of 1.5 gal/h) pulsed on for 3 min at 0700, 1100 and 1500 $_{HR}$. No manual supplemental irrigation was applied.

Study two

Immediately after grafting, plants were placed in a healing chamber in the lab constructed with a vegetable growth shelf (80 inches height, 68 inches length and 18 inches width) covered by one layer of clear plastic. There were three shelves in the chamber representing three blocks, each installed with one LED fixture. Two cool mist humidifiers (Kaz USA, Southborough, MA) were put in the healing chamber to maintain humidity. The whole chamber was manually misted before plants were moved in. Twelve plants for each treatment each block were placed on one layer of capillary mat pre-wetted by 250 ml water, underneath by one layer of clear plastic sheeting. Thereafter, the rooting medium and capillary mat were watered manually when they appeared dry, and the whole chamber was manually misted with 400 ml water each day from 2 to 7 d after grafting in repeat one and every day in repeat two. The temperature was 26±4 °C and relative humidity was 89±6% in the healing chamber during the study period.

Graft healing evaluation

Study one

Immediately after grafting, 10 d and 17 d after grafting, plant height was measured from the soil line to the apical meristem using a ruler, scion length from the graft union to the apical meristem by a ruler, as well as rootstock and scion stem diameter at the mid-point of the rootstock and scion using a caliper. Ten and 17 d after grafting, survival was recorded by rating plants as

living or dead based on the appearance of the scion; plants were regarded as living unless the scion was completely wilted. Each plant was rated by a 0-10 scale for the edema-like physiological disorder; 0 means no edema, and a larger number means more severe edema. Destructive measurement of plant regrowth was taken including leaf and stem fresh weight, as well as leaf and stem dry weight after drying in an oven at 50 °C for 2 d by a balance (MS3002S Precision; Mettler Toledo, Greifensee, Switzerland).

Study two

Immediately after grafting and 5 and 10 d after grafting, plant height, scion length, rootstock and scion stem diameter were measured; 5 and 10 d after grafting, survival, leaf and stem fresh weight and dry weight were recorded, as described in study one.

Digital images were taken of six plants as a unit (two units per treatment per block) immediately after grafting and 5 and 10 d after grafting to monitor leaf area growth. The images of the canopy were taken with a tripod mounted camera (Powershot A2000; Canon USA, Lake Success, NY), and analyzed by the WinCAM software (Regent Instruments, Quebec, Canada), as described before (Hu et al., 2016a). The percentage of leaf area out of the known total area was achieved from the software analysis, and used to calculate the leaf area.

Vascular reconnection through the graft union was monitored 5 d after grafting. Grafted plants were excised from the soil line and placed in an 1 mg/ml Erythrosin B solution. After 15 min, the distance of dye movement was measured from the graft union to the stain terminus (stained length above the graft union).

Data analysis

Relative growth of plant height, scion length, rootstock and scion diameter, and leaf area was calculated as (values 5, 10 or 17 d after grafting-values immediately after grafting)/values immediately after grafting×100%. Compactness was calculated as aboveground dry weight/plant height (mg/cm). Specific leaf area was calculated as leaf area/leaf dry weight (cm^2/g).

Statistical analysis was completed in SAS (version 9.4; SAS Institute, Cary, NC) pooling the data from two repeats in each study. Effects of light intensity treatments were analyzed by the GLIMMIX procedure, with treatment, repeat, block, block*treatment and repeat*treatment as fixed factors in the model. Multiple comparisons among treatments were conducted by the GLIMMIX procedure and its LSMEANS statement with LINES and BYLEVEL options (alpha = 0.05), with the two repeats combined when treatment-repeat interactions were not significant, otherwise, separated.

Results

Study one

All seedlings in study one started to show an edema-like physiological disorder 3 d after grafting in the growth chamber. The disorder characterized as enlarged cells in the vein on the back of the leaves and on the stem (Figure 4B). After plants were moved to the greenhouse, the disorder stopped further development and new leaves grew (Figure 4C).

Six and five of the 11 measured variables were affected by light intensity 10 and 17 d after grafting, respectively ($P < 0.05$) (Table 14). A total of nine and eight parameters differed between repeats 10 and 17 d after grafting, respectively. Light by repeat interactions were significant in plant height and scion diameter relative growth 10 d after grafting and in plant height relative growth 17 d after grafting.

Ten days after grafting, survival was not different among light intensity treatments, all above 90%. Plant height relative growth increased with the increase of light intensity in repeat one, but did not differ among treatments in repeat two (Figure 5). A similar trend was found in scion diameter relative growth, except for the lower value at 150 μmol/m^2/s in repeat two. Leaf fresh and dry weight, stem dry weight and compactness increased with the increase of light intensity, with the data from two repeats combined since there were no significant treatment-repeat interactions in these variables.

Seventeen days after grafting, survival was lower at 5 μmol/m^2/s (85%) than the other three treatments (97.5%) (Figure 6). Edema disorder was most severe at 50 μmol/m^2/s. Plant height relative growth was larger at 300 μmol/m^2/s than the other three treatments in repeat one, but not different among treatments in repeat two. Leaf fresh weight and compactness increased with the increase of light intensity.

Study two

Five days after grafting, all measured parameters including survival, dye movement, and plant regrowth did not differ among treatments (data not shown).

Ten days after grafting, eight out of the 12 survival and plant regrowth variables were affected by light intensity treatments (Table 15). Six parameters differed between the two repeats. The light-repeat interactions were significant in three parameters.

Survival was not affected by the treatments, all above 90%. Leaf area relative growth was larger under the three higher light intensity treatments than that at 5 μmol/m^2/s (Figure 7). Relative growth of plant height and scion length increased with the increase of light intensity in repeat one, but these two parameters and scion diameter relative growth were largest at 150 μmol/m^2/s in repeat two. Leaf fresh and dry weight and compactness increased with the increase of light intensity. Specific leaf area was largest at 50 μmol/m^2/s and smallest at 300 μmol/m^2/s.

Discussion

The reason for the edema-like physiological disorder when grafted plants healed in the plant growth chamber in study one was not clear. It may be caused by some unique conditions in the brand-new chamber. Regardless, light intensity affected wound healing and plant regrowth even when the physiological disorder appeared. To confirm the effects of light intensity on graft healing, study two was conducted in another healing chamber where the physiological disorder did not appear. A similar trend was achieved in study two as that in study one.

In general, survival was not different among treatments except for that at 5 μmol/m^2/s on day 17 in study one, while plant regrowth was larger under higher light intensity from 5 to 300 μmol/m^2/s during the healing period. Two explanations for faster plant regrowth under the higher light intensity may be the increased photosynthesis and photomorphogenesis (Ouyang et al., 2003). A previous study in grafted cucumber found that increased light intensity during the healing process influenced both photosynthetic and morphologic characteristics, and improved photosynthesis, growth, and graft-take (Jang et al., 2011). This suggests that light is an important factor for graft healing in cucumber not only through influencing photosynthesis, but also by regulating morphogenesis.

Higher light intensities up to 300 μmol/m^2/s as tested in this study, may promote photosynthetic rates, and provide energy and substrate for the healing process of grafted plants. In young tomato seedlings, photosynthetic rates and carbon accumulation and exportation increased with the increase of light intensity from 72 to 360 μmol/m^2/s (Nishizawa et al., 2009). Consistent results as improved plant growth under increased light intensities were found in other grafted vegetables. Higher quality of grafted pepper can be obtained at 180 μmol/m^2/s than 50 and 100 μmol/m^2/s (Jang et al., 2013), and higher quality of grafted cucumber at 237 μmol/m^2/s compared to 0 and 142 μmol/m^2/s (Jang et al., 2011). Photosynthesis, plant biomass and leaf area increased while specific leaf area decreased with the increase of light intensity from dark, 50, 98 to 147 μmol/m^2/s in grafted pepper (Jang et al., 2014). Higher light intensity (200 compared to 100 and 0 μmol/m^2/s) enhanced transpiration and photosynthesis, and improved healing and subsequent growth in grafted watermelon (Kim et al., 2005). Increased protein expression related to vascular connection, biomass and graft union hardness was found in watermelon grafted on bottle gourd under higher light intensity at 100 μmol/m^2/s compared to 25, 50, and 75 μmol/m^2/s during healing (Muneer et al., 2015).

Light intensity may also influence light signaling, and then regulate plant development, differentiation, and growth during the graft healing process. Graft union development involves

cellular communication, as well as cell division and differentiation, resulting in callus formation and then vascular re-connection between the rootstock and the scion (Aloni et al., 2008, 2010; Yin et al., 2012). The graft healing process is modulated by phytohormones, especially auxins and cytokinins, which are biosynthesized and translocated under the regulation of light (Neff et al., 2006). Therefore, light may play an important role in wound healing through photomorphogenesis, as suggested in previous studies. Light intensity affected callus growth and differentiation (Afshari et al., 2011; Seibert et al., 1975). Light increased the activities of amine oxidase associated with mechanical wound healing in pea (Petřialský et al., 2007). Light was required for accumulation of proteinase inhibitor in response to insect or mechanical wound in tomato leaves (Green and Ryan, 1973).

Interestingly, in study two, survival, dye movement as the indicator of vascular connection, and plant regrowth did not differ 5 d after grafting. The increased plant regrowth under higher light intensities was shown 10 d after grafting. These results might provide clues about how light intensity affects graft healing, whether by regulating morphogenesis before healing and/or by increasing photosynthesis after healing. Further research is required before we can determine the underlying mechanisms.

A different trend was observed in the relative growth of plant height, scion length, and scion diameter in repeat two of study two where values of these variables were largest at 150 $\mu mol/m^2/s$. The light requirement varies with genotypes, growth status of plants, positions and ages of leaves on a plant (Peat, 1970). Plants in repeat two of study two may reach the photosynthesis saturation point at a lower light intensity than plants from the other repeat and study one. The difference may be caused by different growth conditions during each repeat and thus different plant growth status. Or this different trend may be caused by the confounding effects of light intensity on plant growth and graft healing, since plant elongation is inhibited under higher light intensity.

The results that 150 and 300 μmol/m^2/s promoted plant regrowth questioned current practices for grafted-vegetable propagation. In the U.S., grafted tomato seedlings are generally placed in darkness or dim conditions during the first few days after grafting, and continued in reduced light by gradually increasing light intensity and duration during the first one to two weeks after grafting until the graft union heals (Barrett et al., 2012). Johnson et al. (2011) and Oda (1999) suggested that limited light is needed during healing to reduce photosynthesis and water loss. Rivard and Louws (2006) recommended blocking total sunlight using black plastic. The above recommendation of lower light intensity may be beneficial for graft healing when excessive heat accumulation or water loss would occur under certain environmental conditions with light exposure. However, the results from this study suggest that exposure to 150 to 300 μmol/m^2/s light intensity can promote the growth resumption of newly grafted tomato seedlings when relative humidity and temperature can be controlled within appropriate levels. This can be used as the guidance for light management in the practical propagation of grafted tomatoes.

The air temperature in this study was controlled to be consistent in all treatments using growth chambers and LED lights. The plant temperature may vary under different light intensities. The difference in plant temperature, however, is likely to be marginal and its effects on the measured variables were not tested here. Further studies could include both light intensity and temperature in the treatments to determine the optimal light by temperature combination for the healing of grafted plants.

In conclusion, higher light intensities from 5 to 300 μmol/m^2/s provided solely by LED fixtures tended to enhance plant regrowth of tomato seedlings 10 d after grafting. The results indicated that completely dark or heavily shaded conditions during the graft healing period may not be the optimal light management for grafted tomato propagation. Increased light exposure during graft healing should be considered as long as temperature and relative humidity can be still controlled within the appropriate levels.

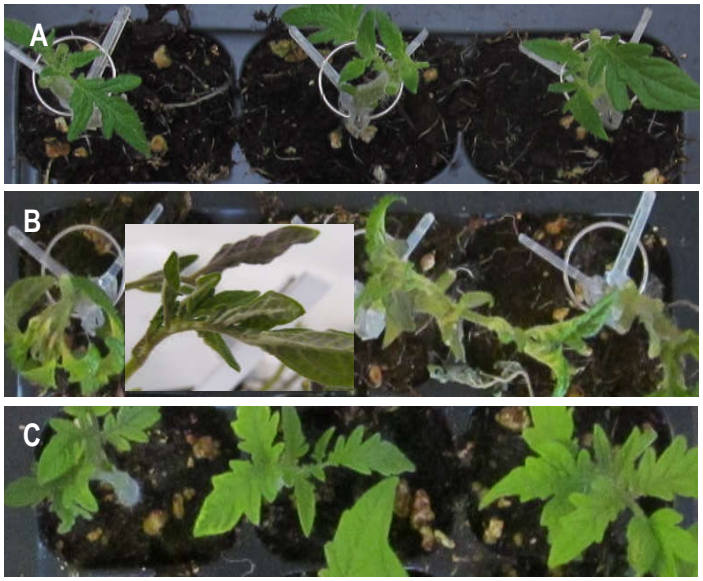

Figure 4. Grafted tomato plants in study one. A, immediately after grafting; B, 10 d after grafting, healed in the growth chamber; insertion shows the edema-like physiological disorder; C, 17 d after grafting, healed in the growth chamber for the first 10 d and then in the greenhouse for 7 d

Table 14. *P* values of Type III tests of the effects of light intensity, repeat and their interaction on survival, edema and plant regrowth 10 d after grafting in the growth chamber, and 17 d after grafting with the first 10 d in the growth chamber and then 7 d in the greenhouse in study one

Source of variance	Survival	Edema	Plant ht relative growth (%)[z]	Scion length relative growth (%)	Rootstock diam relative growth (%)	Scion diam relative growth (%)	Leaf fresh wt (g)[y]	Stem fresh wt (g)	Leaf dry wt (g)	Stem dry wt (g)	Compact-ness (mg/cm)[x]
10 d after grafting (in the growth chamber)											
Light	0.2	0.2	0.0002	0.09	0.6	0.03	0.007	0.8	0.003	0.02	0.01
Repeat	0.3	<.0001	0.0001	0.006	<.0001	0.3	0.04	0.03	0.0007	0.002	0.02
Light×Repeat	0.2	0.6	0.01	0.7	1	0.001	0.5	0.9	0.8	1	0.8
17 d after grafting (the first 10 d in the growth chamber and then 7 d in the greenhouse)											
Light	0.03	0.03	0.004	0.1	0.5	0.3	0.01	0.7	0.1	0.2	0.02
Repeat	0.01	<.0001	0.01	<.0001	<.0001	0.0001	0.4	0.2	0.04	0.05	0.1
Light×Repeat	0.2	0.6	0.03	0.08	0.8	0.06	0.7	0.8	0.4	0.7	0.9

[z] Relative growth = (values 10 or 17 d after grafting - values immediately after grafting)/values immediately after grafting × 100%.
[y] 1 g = 0.0353 oz.
[x] Compactness = aboveground dry weight/plant height. 1 mg = 3.5274 x 10^{-5} oz. 1 cm = 0.3937 inch.

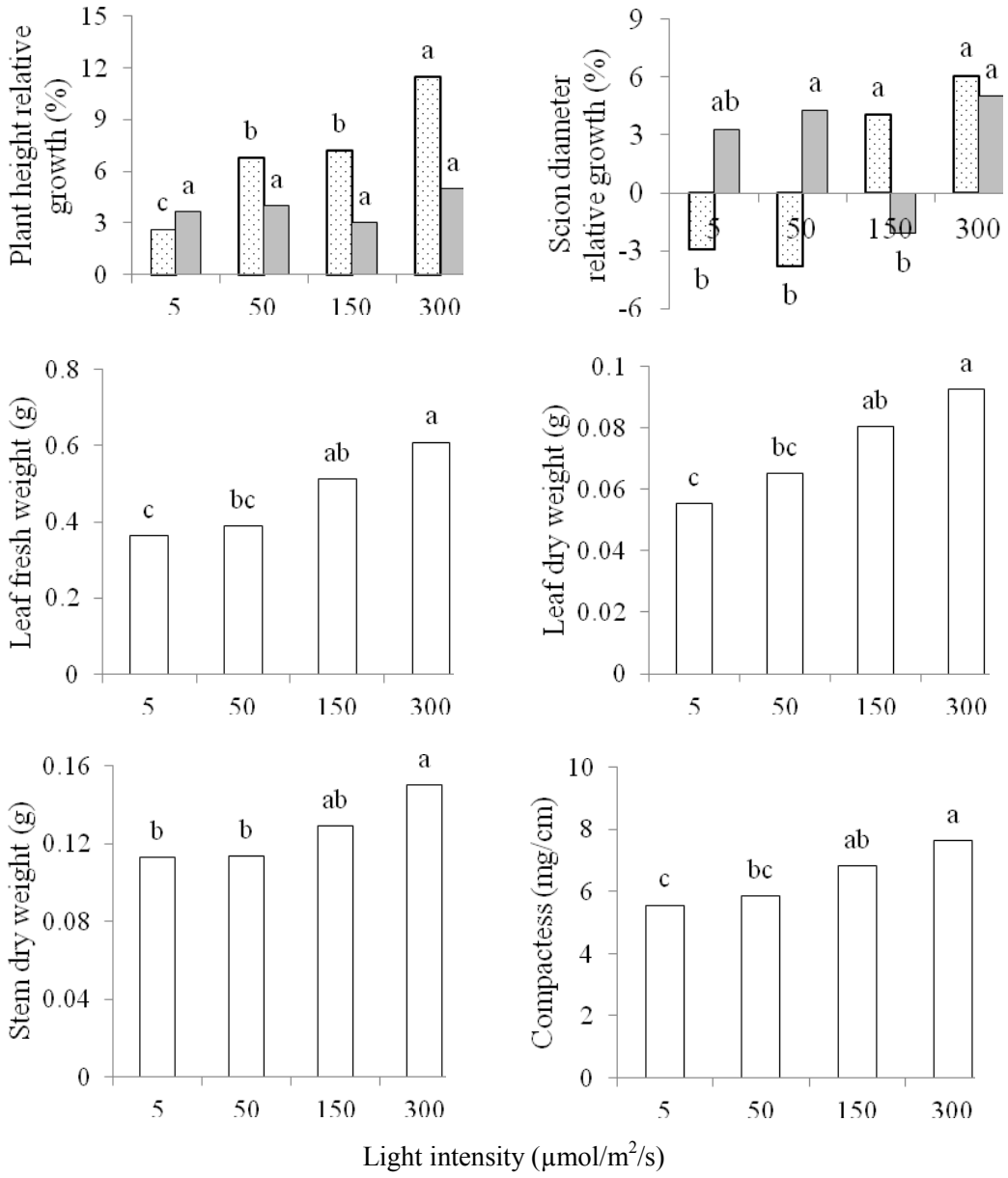

Figure 5. Relative growth of plant height and scion diameter, leaf fresh and dry weight, stem dry weight and compactness 10 d after grafting in the growth chamber in repeat one (dot bars) and repeat two (gray bars), or two repeats pooled (white bars) in study one. Data of two repeats were separated when the treatment-repeat interactions were significant; otherwise, combined. The same letter above bars within each repeat or the two repeats combined represents not significant difference ($P < 0.05$). 1 g = 0.0353 oz. 1 mg = 3.5274 x 10^{-5} oz. 1 cm = 0.3937 inch

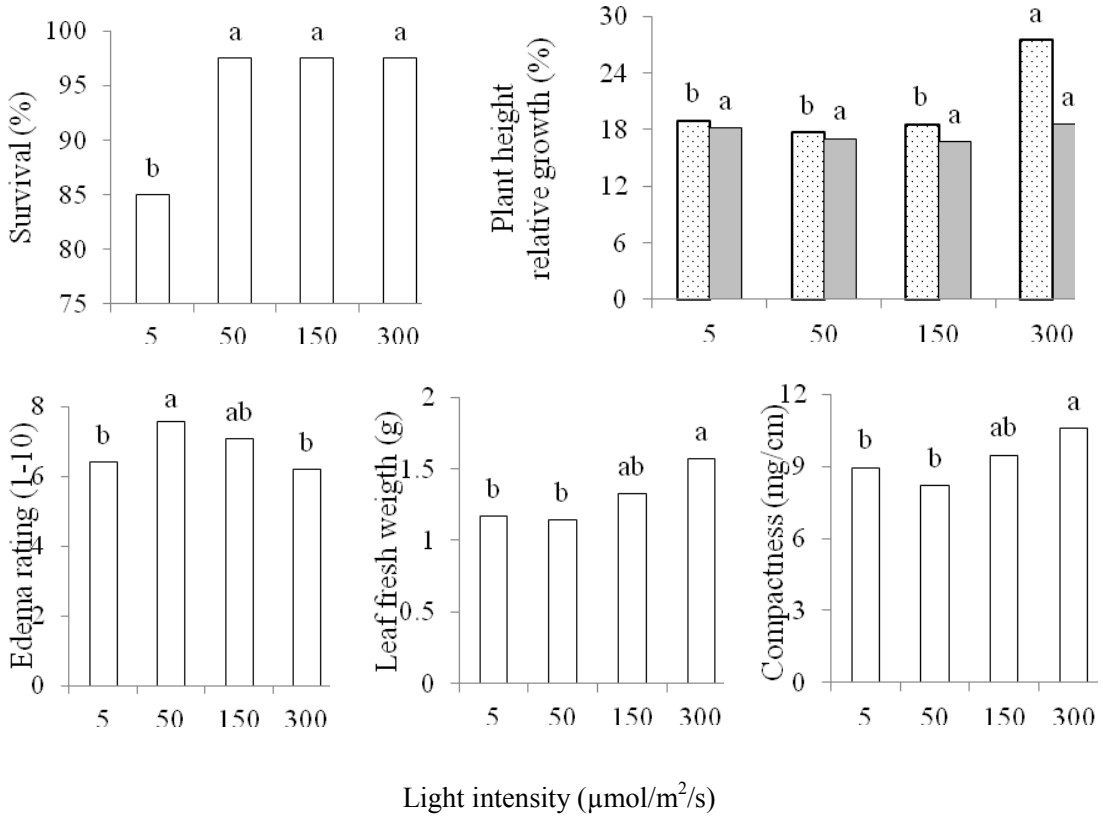

Figure 6. Survival, plant height relative growth, edema, leaf fresh weight and compactness 17 d after grafting with the first 10 d in the growth chamber and then 7 d in the greenhouse in repeat one (dot bars) and repeat two (gray bars), or two repeats pooled (white bars) in study one. Data of two repeats were separated when the treatment-repeat interactions were significant; otherwise, combined. The same letter above bars within each repeat or the two repeats combined represents not significant difference ($P < 0.05$). 1 g = 0.0353 oz. 1 mg = 3.5274 x 10^{-5} oz. 1 cm = 0.3937 inch

Table 15. *P* values of Type III tests of the effects of light intensity, repeat and their interaction on survival and plant regrowth 10 d after grafting in the healing chamber in study two

Source of variance	Survival (%)	Leaf area relative growth (%)[z]	Plant ht relative growth (%)	Scion length relative growth (%)	Rootstock diam relative growth (%)	Scion diam relative growth (%)	Leaf fresh wt (g)[y]	Stem fresh wt (g)	Leaf dry wt (g)	Stem dry wt (g)	Specific leaf area (cm^2/g)[x]	Compactness (mg/cm)[w]
Light	0.7	0.007	<.0001	<.0001	0.05	0.01	0.007	1	0.0002	0.3	0.008	0.002
Repeat	0.3	<.0001	0.005	0.03	0.8	0.4	0.0003	0.4	0.002	0.2	0.9	0.007
Light×Repeat	0.3	0.2	0.01	0.008	0.1	<.0001	0.3	0.9	0.1	0.9	0.8	0.6

[z] Relative growth = (values 10 d after grafting - values immediately after grafting)/values immediately after grafting × 100%.
[y] 1 g = 0.0353 oz.
[x] Specific leaf area = leaf area/leaf dry weight. 1 cm^2 = 0.1550 $inch^2$.
[w] Compactness = aboveground dry weight/plant height. 1 mg = 3.5274 x 10^{-5} oz. 1 cm = 0.3937 inch.

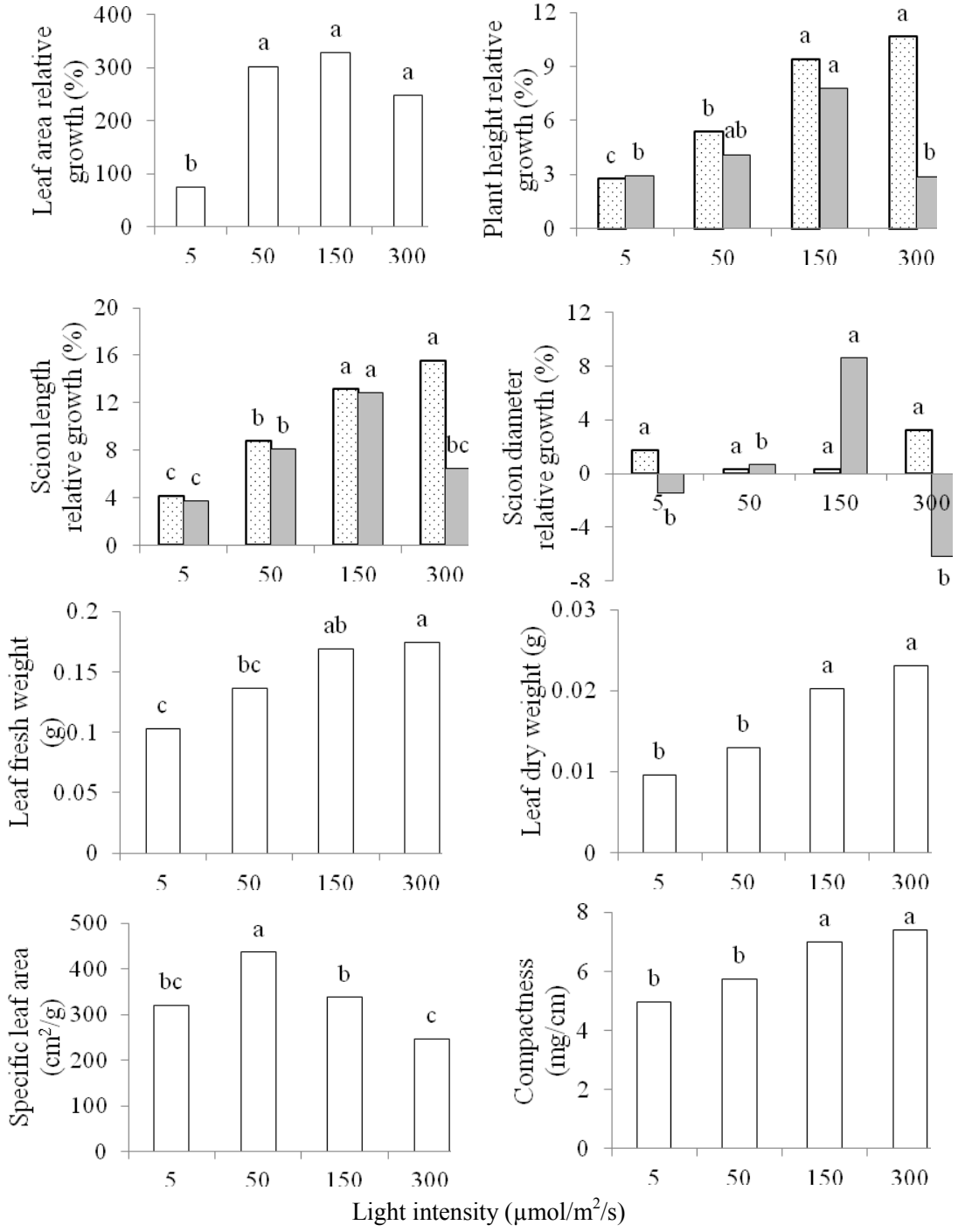

Figure 7. Relative growth of leaf area, plant height, scion length, and scion diameter, leaf fresh and dry weight, specific leaf area and compactness 10 d after grafting in repeat one (dot bars) and repeat two (gray bars), or two repeats pooled (white bars) in study two. Data of two repeats were separated when the treatment-repeat interactions were significant; otherwise, combined. The same letter above bars within each repeat or the two repeats combined represents not significant difference ($P < 0.05$). 1 g = 0.0353 oz. 1 cm^2 = 0.1550 $inch^2$. 1 mg = 3.5274 x 10^{-5} oz. 1 cm = 0.3937 inch

Chapter 5: Temperature and Light Intensity during the Healing Period Affect Survival and Plant Regrowth of Grafted tomato seedlings

Introduction

Grafting has been used to improve vegetable production by combining desirable traits from two plants (the rootstock and scion) into one grafted plant. This technique can effectively manage soilborne diseases (Rivard et al., 2012), overcome abiotic stresses such as extreme temperature, salinity and flood (Schwarz et al., 2010), and enhance water- and nitrogen-use efficiency (Djidonou et al., 2013, 2015). Therefore, grafted plants can out-perform ungrafted ones and increase productivity.

However, the grafting process creates severe wound where plants are cut. Complete healing to resume vascular functioning between the rootstock and the scion and plant growth is essential for graft success, which takes about 8 d in tomato (Fernández-García et al., 2004). During the healing period, careful environmental management is required to ensure graft union development and plant regrowth.

Light and temperature are likely to influence graft healing. The effects of light and temperature on graft healing may be related to complex physiological pathways, especially through regulating photosynthesis and transpiration in newly grafted plants. Both light and temperature are the major factors for modulating carbon assimilation and have a close correlation in regulating photosynthesis (Berry and Björkman, 1980). Net photosynthesis at constant temperature first increased from low light intensity to the saturation point and then remained stable above the light saturation point, whereas net photosynthesis at saturated light first increased

from low to optimal temperature and then declined above the optimal temperature. Light saturation point increases with the increase of temperature when other conditions are the same. On the other hand, these two environmental factors also affect plant water status by regulating transpiration, which is important for newly grafted plants where water transport from roots to shoots terminates before healing. Transpiration at a constant temperature increased with the increase of light intensity and stabled at the light saturation point. Transpiration at constant absolute humidity and saturating light intensity increased with the increase of temperature up to the experimental maximum (Crane et al., 1983; Kim et al., 2013). For newly grafted seedlings, increased photosynthesis may benefit healing by providing energy. However, excessive transpiration can lead to wilted plants and even death when the grafted seedlings have no vascular connection and no water transportation to the shoots before graft healing. Therefore, a balance of photosynthesis and transpiration regulated by the optimal combination of light and temperature conditions may promote graft healing.

However, research-based information on the optimal light and temperature combination during the healing of grafted tomato seedlings is unavailable. Wound-induced accumulation of proteinase inhibitor is light- and temperature-dependent in young tomato leaves (Green and Ryan, 1973). A few previous studies suggested that relatively higher light intensity after grafting can promote the healing process (Jang et al., 2011, 2013, 2014). The optimal temperature for healing of grafted tomato seedlings is still unclear. Growth of grafted cacti was greater at 25 °C during the healing period compared to that at 30 and 35 °C (Jeong et al., 2007). Oda (2007) suggested that temperature maintained at 28 to 30 °C was proper for rapid healing of generally grafted vegetables. For tomato, the range of temperature recommended for graft healing is 21-29 °C according to De Ruiter Seeds (2006). A negative difference between day and night temperatures during the healing period for grafted tomato plants may prevent excessive stem elongation (Ito et al., 1995). To our knowledge, no previous reports included both light and temperature factors for

graft healing of tomato. Masterson et al. (2016) suggested that light-temperature interactions on graft healing be studied thoroughly.

The lack of information on the separate and interactive effects of light intensity and temperature during the healing period largely hinders the optimization of environmental management for efficient propagation of grafted vegetables. Current practices for healing grafted vegetables are inconsistent and questionable. In Asian, partially shading is applied to grafted plants during healing and acclimatization to avoid unfavorable high temperature (Lee et al., 2010). In Europe, reduction of radiation to about 60-70% is applied until plants heal to avoid excessive heat, but excessive shading is prevented since a lack of light can inhibit assimilation and produce weak seedlings (Leonardi and Romano, 2002). In the U.S. grafted vegetables are commonly healed in enclosed structures shaded to reduce light levels and moderate temperature (Rivard and Louws, 2006; Rivard and Louws, 2011). Shade cloth with 27% light transmission was used to cover healing chambers to create dim light conditions (Johnson and Miles, 2011), or two layers of shade cloth or one layer of black plastic was used to create dark conditions during the first few days of healing (Johnson et al., 2011). What is the range of temperature grafted tomato can tolerate during the healing period? How much shade is necessary to achieve the target temperature if no other approaches are available for temperature control? What is the optimal combination of light and temperature for graft healing if light and temperature can be controlled individually? Answers to these questions will provide guidance for optimal environmental management during the healing period for grafted plants.

Determining the optimal conditions which improve the propagation of grafted vegetables is profoundly important. The current prices for grafted transplants are much higher than the prices for their ungrafted counterparts, which has become the barrier to a wider use of grafted plants. The cost of post-grafting care is a significant factor in price determination (Lee et al., 2010; Rivard et al., 2010). With the optimization of conditions during the healing period, the efficiency

of grafted-plant propagation will increase and thus the prices of grafted transplants will decrease to facilitate the application of grafting to vegetable production.

The hypothesis was that light and temperature had separate and interactive effects on the survival and regrowth of grafted tomato plants. The objectives were to 1) test the survival and regrowth of grafted tomato seedlings under different combinations of temperature and light intensity conditions; 2) heighten the understanding of the effects of these two key environmental factors during the healing period; and 3) eventually optimize the environmental management for grafted tomato propagation.

Materials and Methods

Two studies were conducted at the Ohio Agricultural Research and Development Center in Wooster, OH. Study one was conducted with five replications over time from 6 Apr. 2015 to 4 July 2015, and study two with three replications over time from 3 Mar. 2016 to 2 May 2016.

Plant materials and seedling preparation

Tomato rootstock 'Maxifort' and tomato scion 'Cherokee Purple' were used. Seedlings were grown in a greenhouse with high-intensity discharge lights came on at dawn and stayed on for 16 h unless outdoor sunlight stayed above 350 W/m^2 for 20 min. The temperature was set at 22-26 °C from dawn to dusk and 17-21 °C from dusk to dawn in study one, and 25-28 °C from dawn to dusk and 20-23 °C from dusk to dawn in study two. Seed was sown in 96-cell trays preloaded with growing medium (Pro-Mix® MP Mycorrhizae™ Organik™; Premier Tech, Quebec, Canada). Trays were placed on a capillary mat (Kapmat; Buffalo Felt Products Corp, West Seneca, NY) underlain by clear plastic. Trays were hand-misted to wetness immediately after sowing; an automated irrigation system was used thereafter for 1 week to maintain soil moisture, for each tray, with 1 fogger (each delivered a flow rate of 8.1 gal/h) pulsed on for 8 s every 30 min every day, and 2 drippers (each with a flow rate of 1.5 gal/h) pulsed on for 3 min at 0700, 1100 and 1500 $_{HR}$ every day in study one and for 4 min at 0700, 1100, 1500 and 1900 $_{HR}$ every

day in study two. One week after seed sowing, no automatic irrigation was applied and seedlings were hand-misted overhead once to twice a day depending on the weather.

Each seedling was marked by the day of emergence. One day before grafting, seedlings were sorted based on emergence dates and plant sizes to ensure matching in stem diameter between rootstock and scion when grafted.

Grafting procedure

Three to four weeks after seeding, 'Cherokee Purple' were grafted to 'Maxifort' using the splice grafting method as described by Hu et al. (2016b). 'Maxifort' were decapitated right below the cotyledons at a 45° angle, and 'Cherokee Purple' were derooted where the stem diameter matched with that of the rootstock at a 45° angle. The two portions were put together and secured with a spring grafting clip. Largest leaves were trimmed from the scion remaining a consistent leaf area on each grafted plants.

Temperature and light treatments during healing

Immediately after grafting, plants were put on one layer of a pre-wetted capillary mat in a bottom tray and placed in environmental-controlled growth chambers (Canviron; Controlled Environments Ltd., Manitoba, Canada) for healing. In study one, grafted plants were healed under two temperature (25/20 and 30/25 °C, day/night) by two light intensities (50 and 150 μmol/m^2/s). In study two, grafted plants were healed under three temperature (15/25, 25/25 and 35/25 °C, day/night) by two light intensities (150 and 300 μmol/m^2/s). Fifteen to eighteen plants were used for each treatment per replication in study one and twenty plants in study two.

Temperature and light intensity treatments were arranged in growth chambers as a split-plot design. Temperature treatments were randomized in different growth chambers as the main plot factor. In each growth chamber, two zones differing in light intensity were created as the sub-plot factor by varying the distance between light sources and plants and using open frames covered with a black knitted shade cloth (50% PAR transmission; Tek Inc., Janesville, WI). The light in all growth chambers was provided from one 400-W metal halide lamp (GE Lighting, Inc.,

East Cleveland, OH) and one 400-W high-pressure sodium lamp (GE Lighting, Inc., East Cleveland, OH). Photoperiod was 12 h from 0700 to 1900 $_{HR}$ in all treatments.

Relative humidity in study one was controlled at 90% for all treatments for the first 7 d and reduced to 80% on the 8th d and 60% on the 9th and 10th d. Relative humidity in study two was set at 100% under all treatments for the first 3 d, and lowered to 80% at 15 °C, 90% at 25 °C, 94% at 35 °C for 4th to 7th d, and 45% at 15 °C, 70% at 25 °C, 83% at 35 °C for 8th to 10th d, to achieve similar vapor pressure deficit among treatments. Temperature and relative humidity conditions were monitored hourly throughout the study using data loggers (Hobo ProV2 version 2.5.0; Onset Computer Co., Pocasset, MA). Light intensities were monitored daily by a light meter (LI-250A; LI-COR Inc., Lincoln, NE). Water was added to the rooting medium and capillary mat manually when they appeared dry.

Plant regrowth measurement

Nondestructive measurement of plant regrowth was taken immediately after grafting and 10 d after grafting. Digital images of plants were taken to monitor leaf area as described before (Hu et al., 2016a). The images were analyzed by the WinCAM software to separate colors of leaves from the background and calculate the leaf area. Plant height was measured from the soil line to the meristem and scion length from the graft union to the meristem by a ruler. Stem diameter was measured at the midpoint of the rootstock and the scion by a caliper.

Destructive measurement of plant regrowth was taken 10 d after grafting. Leaf and stem fresh weight as well as leaf and stem dry weight after drying in an oven at 50 °C for 2 d were measured by a balance (MS3002S Precision; Mettler Toledo, Greifensee, Switzerland).

Relative growth of leaf area, plant height, scion length, as well as rootstock and scion diameter were calculated as (values 10 d after grafting – values immediately after grafting)/values immediately after grafting × 100%. Specific leaf area was calculated as leaf area/leaf dry weight (cm^2/g). Compactness was calculated as aboveground dry weight/plant height (mg/cm).

Data analysis

Statistical analysis was completed in SAS (version 9.4; SAS Institute, Cary, NC). Separate and interactive effects of temperature and light were analyzed by the GLIMMIX procedure, with temperature, light, and temperature by light interaction as the fixed factors, and replication and replication by temperature interaction as the random factors. Multiple comparisons among treatments were conducted using the LSMEANS statement with LINES and BYLEVEL options in GLIMMIX.

Results

Study one

Temperature alone did not affect survival and plant regrowth in study one ($P < 0.05$) (Table 16). Light intensity significantly affected ten out of the 12 variables measured. The interactions between temperature and light intensity were significant in three out of the 12 parameters.

Ten days after grafting, the main effects of light intensity showed that150 versus 50 μmol/m^2/s achieved larger relative growth of plant height, scion length, scion diameter and leaf area, leaf and stem fresh and dry weight as well as compactness, and smaller specific leaf area (Table 17). Multiple comparisons among the four temperature-light combinations showed that survival and rootstock diameter relative growth did not differ. Scion diameter and leaf area relative growth, leaf fresh weight, leaf and stem dry weight and compactness were larger under 150 versus 50 μmol/m^2/s, without significant temperature by light interactions. Specific leaf area was larger under 50 versus 150 μmol/m^2/s regardless of temperature. Relative growth of plant height and scion length followed a similar trend as largest at 30/25 °C under 150 μmol/m^2/s, followed by 25/20 °C under 150 μmol/m^2/s, and smallest under 50 μmol/m^2/s regardless of temperature. Stem fresh weight was largest under 150 μmol/m^2/s regardless of temperature, followed by that at 25/20 °C under 50 μmol/m^2/s, and smallest at 30/25 °C under 50 μmol/m^2/s.

In general, under 150 μmol/m^2/s, the higher temperature at 30/25 °C achieved numerically or significantly larger plant height, scion length, and stem fresh weight compared to the lower temperature at 25/20 °C; whereas, under 50 μmol/m^2/s, the higher temperature at 30/25 °C achieved numerically or significantly smaller plant height, scion length, and stem fresh weight.

Study two

Temperature and light intensity significantly affected five and six out of the ten variables measured in study two, respectively (Table 18). The interactions of temperature and light intensity were significant in seven out of the ten parameters.

Ten days after grafting, survival, aboveground dry weight, and compactness were larger at 25/25 °C than the other two temperatures (Table 19). Rootstock diameter relative growth and specific leaf area were larger at 35/25 °C. Relative growth of plant height and scion length, and specific leaf area were larger under 150 versus 300 μmol/m^2/s, while rootstock diameter relative growth, aboveground dry weight, and compactness were larger under 300 μmol/m^2/s. Multiple comparisons among the six temperature-light combinations showed that survival was highest at 25/25 °C regardless of light intensities and at 35/25 °C under 150 μmol/m^2/s than the other temperature-light combinations. Relative growth of plant height and scion length was significantly or numerically largest at 35/25 °C under 150 μmol/m^2/s than the other temperature-light combinations. Relative growth of rootstock diameter was larger at 35/25 °C under 300 μmol/m^2/s. Aboveground dry weight and compactness were largest while specific leaf area was smallest at 25/25 °C under 300 μmol/m^2/s.

Discussion

Temperature ranging from 25/20 to 30/25 °C as tested in study one, in general, did not affect survival or plant regrowth of grafted tomato seedlings, while 15/25 °C and 35/25 °C as tested in study two resulted in lower survival, aboveground dry weight and compactness compared to 25/25 °C. This result is consistent with the previous reports. Grafted tomatoes had

above 90% survivorship under temperature (23-25 °C) fluctuation in different healing chamber designs (Johnson and Miles, 2011). Graft survival of tomato was 91-94% under a daily average temperature at 21 °C with minimum and maximum temperatures at 14 and 29 °C; while survival was 77-87% under a daily average temperature at 20 °C with minimum and maximum temperatures at 11 and 34 °C (Masterson et al., 2016). Day temperature from 25 to 30 °C is appropriate during healing for grafted tomato seedlings, and 15 and 35 °C are out of the appropriate temperature range.

The difference between day and night time temperatures (DIF) may also affect graft healing. DIF in study one was the same as +5 °C, but the 30/25 °C treatment had a higher day, night and daily average temperature than the 25/20 °C treatment. Higher day temperature may enhance gross photosynthesis and wound healing, but the higher night temperature may promote respiration, compensating the increased photosynthesis in the daytime. Therefore, the higher day/night temperature treatment did not result in larger plant growth. However, Hussey (1965) found that during the first 6 weeks of growth of young tomato seedlings, higher night temperature within the range of 15-25 °C did not significantly increase respiratory loss in dry weight, instead, increased leaf area and thus photosynthetic surface in the daytime. The optimal day and night temperatures for tomato seedling growth are both close to 25 °C. The two temperature treatments tested in study one, one with the optimal night temperature, and the other with the optimal day temperature, did not cause a difference in plant regrowth of newly grafted tomatoes. In study two, DIF was -10, 0, and +10 °C in the three temperature treatments. Negative DIF reduced young tomato plant growth and day temperature played a bigger role than night temperature in affecting plant growth (Heuvelink, 1989). Results from study two were consistent with the previous findings. The treatment with positive DIF at 35/25 °C tended to have larger growth of plant length and stem diameter. The treatment at 25/25 °C achieved larger survival, aboveground dry weight and compactness possibly due to the optimum day and night temperatures at 25 °C for tomato seedlings.

Higher light intensity generally promoted graft healing and plant regrowth at 150 μmol/m^2/s compared to 50 μmol/m^2/s in study one, and enhanced rootstock diameter relative growth, aboveground dry weight and compactness under 300 versus 150 μmol/m^2/s in study two. This result was consistent with previous studies (Jang et al., 2011, 2013, 2014), suggesting that light exposure during the healing period may promote healing of grafted tomato seedlings. The common recommendation of low light intensity during the first few days after grafting for healing of grafted vegetables (Davis et al., 2008) may need to be reconsidered. In addition, the high and low light intensities supplied by metal halide and high-pressure sodium lamps may modify the leaf temperature, which could be further studied in the future. The temperature treatments of the current study considered only the air temperature around plant tissues, but the results are applicable to practical operations since propagators usually only monitor and modify the air temperature rather than plant temperature.

Interactions between temperature and light intensity were significant in some parameters of survival and plant regrowth. In study one, under the higher light intensity at 150 μmol/m^2/s, the higher temperature at 30/25 °C versus 25/20 °C tended to achieve larger plant regrowth; under the lower light intensity at 50 μmol/m^2/s, the lower temperature at 25/20 °C versus 30/25 °C tended to achieve larger plant regrowth. These trends were consistent with the common understanding that optimal temperature for plant growth increased with the increase of light intensity (Went, 1945). In study two, survival was higher under the moderate temperature regardless of light intensities or the higher temperature with the lower light intensity. Vu et al. (2014) reported that 23 °C achieved higher survival rates of grafted tomato seedlings compared to 17, 20 and 26 °C under 30 μmol/m^2/s light intensity from fluorescent lamps. The higher temperature with positive DIF and the lower light intensity increased plant elongation, the higher temperature with positive DIF and the higher light intensity increased stem diameter, and the moderate temperature with the higher light intensity increased biomass and compactness. The moderate temperature at 25/25 °C

under the higher light intensity at 300 μmol/m^2/s seemed to be the best for graft healing among the tested combinations considering all the measured parameters.

These results suggest that the common practice of grafted-plant propagation with dim or dark conditions need to be reconsidered, provided humidity and temperature control is reliable. Grafted tomato plants seem to be flexible for temperature from 25/20 to 30/25 °C, and benefit from higher light intensities during the first 10 days after grafting. Therefore, the appropriate temperature during the healing period can be ranged from 25/20 to 30/25 °C, and the favorable range of light intensity can be extended up to 300 μmol/m^2/s under the moderate temperature at 25/25 °C, under the high humidity as used here. However, day temperature at 35 °C under 300 μmol/m^2/s reduced survival, and shading may be necessary to avaoid excessive heat built up if alternative temperature control is unavailable.

Table 16. *P* values of type III tests for effects of temperature and light and their interaction on survival and plant regrowth of grafted tomato seedlings 10 d after grafting in study one

Source of variance	Survival (%)	Plant ht relative growth (%)[z]	Scion length relative growth (%)	Rootstock diam relative growth (%)	Scion diam relative growth (%)	Leaf fresh wt (g)[y]	Stem fresh wt (g)	Leaf dry wt (g)	Stem dry wt (g)	Compact-ness (mg/cm)[x]	Leaf area relative growth (%)	Specific leaf area (cm^2/g)[w]
Temp	0.9	0.5	0.3	0.6	0.4	0.5	0.3	0.2	1.0	0.4	0.8	0.3
Light	0.08	<.0001	<.0001	0.3	<.0001	<.0001	<.0001	<.0001	0.002	<.0001	0.03	<.0001
Interaction	0.5	<.0001	<.0001	0.4	0.5	0.3	0.02	0.2	0.5	0.6	0.2	0.05

[z] Relative growth = (values 10 d after grafting - values immediately after grafting)/values immediately after grafting × 100%.

[y] 1 g = 0.0353 oz.

[x] Compactness = aboveground dry weight/plant height. 1 mg = 3.5274 x 10^{-5} oz. 1 cm = 0.3937 inch.

[w] Specific leaf area = leaf area/leaf dry weight. 1 cm^2 = 0.1550 $inch^2$.

Table 17. Survival and plant regrowth of grafted tomato seedlings 10 d after grafting under two temperature and two light intensity conditions during the healing period in study one

Factor		Survival (%)	Plant ht relative growth (%)[z]	Scion length relative growth (%)	Rootstock diam relative growth (%)	Scion diam relative growth (%)	Leaf fresh wt (g)[y]	Stem fresh wt (g)	Leaf dry wt (g)	Stem dry wt (g)	Compact-ness (mg/cm)[x]	Leaf area relative growth (%)	Specific leaf area (cm^2/g)[w]
Temp (day/night °C)													
25/20		95a[v]	16a	25a	3a	7a	0.20a	0.42a	0.02a	0.03a	7a	589a	380a
30/25		95a	17a	29a	2a	5a	0.19a	0.40a	0.02a	0.03a	7a	611a	342a
Light (ìmol/m^2/s)													
50		93a	12b	20b	2a	4b	0.17b	0.38b	0.01b	0.02b	6b	524b	434a
150		98a	22a	35a	3a	8a	0.22a	0.44a	0.03a	0.03a	8a	676a	287b
Temp (day/night °C)	Light (imol/m^2/s)												
25/20	50	93a	13c	21c	2a	4bc	0.18bc	0.40b	0.01b	0.02bc	5b	554ab	471a
	150	96a	19b	30b	4a	9a	0.22a	0.44ab	0.02a	0.03ab	8a	625ab	288b
30/25	50	92a	10c	18c	2a	3c	0.16c	0.35c	0.01b	0.02c	6b	495b	398a
	150	99a	25a	41a	2a	7ab	0.22ab	0.45a	0.03a	0.03a	8a	728a	286b

[z] Relative growth = (values 10 d after grafting - values immediately after grafting)/values immediately after grafting × 100%.
[y] 1 g = 0.0353 oz.
[x] Compactness = aboveground dry weight/plant height. 1 mg = 3.5274×10^{-5} oz. 1 cm = 0.3937 inch.
[w] Specific leaf area = leaf area/leaf dry weight. 1 cm^2 = 0.1550 $inch^2$.
[v] Means under each category followed by the same letter are not significantly different ($P < 0.05$).

Table 18. *P* values of type III tests for effects of temperature and light and their interaction on survival and plant regrowth of grafted tomato seedlings 10 d after grafting in study two

Source of variance	Survival (%)	Plant ht relative growth (%)[z]	Scion length relative growth (%)	Rootstock diam relative growth (%)	Scion diam relative growth (%)	Aboveground fresh wt (g)[y]	Aboveground dry wt (g)	Compact-ness (mg/cm)[x]	Leaf area relative growth (%)	Specific leaf area (cm^2/g)[w]
Temp	0.02	0.1	0.1	0.005	0.2	0.5	0.03	0.009	0.4	0.05
Light	0.2	<.0001	<.0001	0.02	0.1	0.5	0.002	0.0001	0.2	<.0001
Interaction	0.0004	<.0001	<.0001	0.001	0.4	0.2	0.03	0.04	0.06	0.01

[z] Relative growth = (values 10 d after grafting - values immediately after grafting)/values immediately after grafting × 100%.
[y] 1 g = 0.0353 oz.
[x] Compactness = aboveground dry weight/plant height. 1 mg = 3.5274×10^{-5} oz. 1 cm = 0.3937 inch.
[w] Specific leaf area = leaf area/leaf dry weight. 1 cm^2 = 0.1550 $inch^2$.

Table 19. Survival and plant regrowth of grafted tomato seedlings 10 d after grafting under three temperature and two light intensity conditions during the healing period in study two

Factor		Survival (%)	Plant ht relative growth (%)[z]	Scion length relative growth (%)	Rootstock diam relative growth (%)	Scion diam relative growth (%)	Aboveground fresh wt (g)[y]	Aboveground dry wt (g)	Compact-ness (mg/cm)[x]	Leaf area relative growth (%)	Specific leaf area (cm^2/g)[w]
Temp (day/night °C)											
15/25		84b[v]	13a	22a	9b	21a	0.46a	0.044b	8.4b	71a	229ab
25/25		98a	21a	37a	12b	25a	0.52a	0.054a	9.6a	104a	203b
35/25		89b	23a	40a	20a	29a	0.49a	0.044b	7.6b	88a	277a
Light (ìmol/m^2/s)											
150		91a	21a	37a	13b	24a	0.49a	0.045b	8.1b	93a	258a
300		89a	17b	29b	15a	26a	0.49a	0.049a	8.9a	82a	214b
Temp (day/night °C)	Light (ìmol/m^2/s)										
15/25	150	78c	14bc	24bc	10cd	21b	0.46a	0.042c	8.3bc	72a	237b
	300	90b	12c	20c	8d	22ab	0.47a	0.045bc	8.5bc	70a	222b
25/25	150	98a	22ab	36ab	11cd	22ab	0.52a	0.050b	8.8b	101a	229b
	300	97ab	21ab	37ab	13c	27ab	0.52a	0.058a	10.3a	107a	176c
35/25	150	97ab	28a	49a	17b	28a	0.50a	0.043c	7.3d	106a	309a
	300	82c	18bc	30bc	23a	29a	0.47a	0.044bc	7.9cd	70a	246b

[z] Relative growth = (values 10 d after grafting - values immediately after grafting)/values immediately after grafting × 100%.
[y] 1 g = 0.0353 oz.
[x] Compactness = aboveground dry weight/plant height. 1 mg = 3.5274 x 10^{-5} oz. 1 cm = 0.3937 inch.
[w] Specific leaf area = leaf area/leaf dry weight. 1 cm^2 = 0.1550 $inch^2$.
[v] Means under each category followed by the same letter are not significantly different ($P < 0.05$).

Chapter 6: Fertilization and Grafting Effects on Tomato Plant Growth, Yield, and Fruit Quality in Conventional, Open Field Production

Introduction

Grafting has been an emerging technology used for tomato production. Previous reports have demonstrated that grafting can improve vegetable productivity through resistance to diseases (King et al., 2008; Louws et al., 2010) as well as tolerance to flooding (Bhatt et al., 2015), drought (Nilsen et al., 2014; Sánchez-Rodríguez et al., 2014), salinity (He et al., 2009), extreme temperature, and organic pollutant (Schwarz et al., 2010). The major reason for tomato grafting was to overcome biotic and abiotic stresses at the beginning of growers' adopting this technology, while the application of grafting is becoming wider nowadays. Diverse production systems use grafted plants, including large and small scale, conventional and organic, open field and protected growing conditions, with presence or absence of stresses, standard or adjusted management practices.

The performance of grafted plants varies with growing conditions, management, and rootstock-scion combinations (Gajc-Wolska et al., 2015; Kakita et al., 2015; Khah et al., 2006; Kumar et al., 2015; Waiganjo et al., 2013). Grafting does not guarantee improved plant growth, yield, and fruit quality in all situations of tomato production. More information on grafted plant performance with different rootstocks under various conditions is needed for a broader application of grafting (Davis et al., 2008; Kubota et al., 2008).

Nutrient uptake differed among rootstock-scion combinations, which should be taken into account when developing fertilization regimens for particular grafted combinations (Leonardi and

Giuffrida, 2006; Ruiz et al., 1997). Nitrogen-uptake and utilization efficiency were higher in grafted melons than their ungrafted counterparts (Colla et al., 2010; Ruiz and Romero, 1999). The enhanced fertilizer use efficiency in some grafted combinations suggest that fertilization management can be adjusted to prevent yield losses due to marginal soil fertility and fertilizer losses due to leaching (Savvas et al., 2010). Therefore, we questioned how to optimize the fertilization management matching with rootstock-scion combinations to maximize the positive impacts of grafting for tomato production.

There is limited research studying the fertilization management for grafted tomato production. Djidonou et al. (2013) reported higher yield, increased nitrogen use efficiency, and greater yield potential with the increase of nitrogen rates in grafted plants versus ungrafted ones in tomato field production with sandy soils in north Florida. Grafted tomato plants had a lower nitrogen fertilization rate requirement than ungrafted ones to achieve the same yield goal (Djidonou et al., 2015). It is unknown about how different grafted combinations respond to various fertilization regimens under the open field conditions in Ohio. In addition, comprehensive grafted tomato performance including plant vegetative growth, yield components and fruit quality of multiple rootstock-scion combinations under different fertilization treatments is undocumented.

The objective of this study was to test the separate and interactive effects of fertilization and grafting on plant growth, yield, and fruit quality in conventional, open field fresh market tomato production in Ohio. Plant growth was monitored by both destructive and non-destructive measurements. Yield components included weight and number of total and marketable fruit. Tomato fruit quality was monitored by measuring °Brix, pH and titratable acidity (TA). Sugars and acids are the most important components of tomato flavor (Cuartero and Fernández-Muñoz, 1999). High concentrations of both are required for good flavor. °Brix reflects soluble solids (Kleinhenz and Bumgarner, 2013a), and correlates well with sugars. TA and pH reflect acid concentrations in tomato fruit.

Materials and Methods

The study was conducted twice in 2013 and 2014 at The Ohio State University, Ohio Agricultural Research and Development Center in Wooster, Ohio. A split-plot design was used with fertilization treatment as the main plot and rootstock treatment as the subplot. Two fertilization regimens, i.e., pre-plant fertilization only and pre-plant fertilization plus standard fertigation, were arranged in a randomized complete block design with four blocks. Two commercial tomato rootstock cultivars 'Maxifort' and 'Emperador', one experimental tomato rootstock '320', and the ungrafted control were randomized within each main plot. 'BHN 589' was used for the scion and the ungrafted control. Within each main plot, there were three complete sets of subplots; two sets were used for the two vegetative harvests following with destructive plant growth measures, and the third set was used for the non-destructive plant growth measures and fruit harvests. There were five plants in each subplot for a given treatment per set with the three plants in the middle used for data collection.

'BHN 589' seed used for scion was sown on 7, 11 and 15 Mar. in 2013 and on 1, 4 and 8 Apr. in 2014. Rootstock seed was sown on 11 and 15 Mar. in 2013 and on 4 and 8 Apr. in 2014. 'BHN 589' seed used for ungrafted controls was sown on 17 Apr. in 2013 and on 28 Apr. in 2014. Plants were grafted using the cleft grafting method as described in Hu et al. (2016b) on 2, 4 and 9 Apr. in 2013 and on 22, 25 and 29 Apr. in 2014. Grafted plants were completely healed and acclimatized before transplanting. Grafted plants and ungrafted controls were transplanted to raised beds with black plastic and drip irrigation in the open field on 17 May in 2013 and on 3 June in 2014. Beds were 2.5-foot wide and on 5-foot centers with 2-foot in-row spacing between plants for tomato production. Plants were transplanted such that the graft union remained approximately 2.5 cm above the soil line. Plants were trellised using the Florida Weave system beginning 2 weeks after planting and continuing as needed throughout the season.

Pre-plant fertilization was applied to all plots at a rate of 300 lb/A 19-19-19 in 2013 and 400 lb/A 10-20-20 in 2014, and 13 lb nitrogen (N)/A from composted dairy manure in both years.

After transplanting, fertigation was applied to the main plots of the pre-plant fertilization plus standard fertigation treatment. Peters® Excel 15-5-15 CAL-MAG SPECIAL was applied on 27 June and 8, 15, 23 and 30 July at a rate of 10 lb N/A for each application and on 6 Aug. at a rate of 5 lb N/A in 2013. The same fertilizer was applied on 14, 22 and 28 July and 4, 13, 18 and 25 Aug. at a rate of 10 lb N/A for each application in 2014.

Plant growth was monitored by non-destructive and destructive measures. Truss number was counted 4, 6 and 8 weeks after transplanting. Plants were checked once a day to record dates of anthesis of the first flower, appearance of the first green fruit larger than 5 mm in diameter, and appearance of the first red fruit reaching the blush stage. Duration in days of Transplant-Flower (from transplanting to anthesis of the first flower), Flower-Fruit (from anthesis of the first flower to appearance of the first green fruit larger than 5 mm in diameter), and Fruit Ripening (from appearance of the first green fruit larger than 5 mm in diameter to appearance of the first red fruit reaching the blush stage) was calculated. Three plants in the middle of the subplot from each treatment were harvested for the first and second destructive plant growth measurement on 19 June and 19 Aug. in 2013, and on 8 July and 3 Sep. in 2014, respectively. Stem and leaf fresh and dry weight were measured using a scale, leaf area was measured by a leaf area meter, and ratios of stem fresh/dry weight to aboveground fresh/dry weight were calculated. In the second vegetative harvest, leaf nitrogen (NO_3-N) content was also measured by the HORIBA CARDY Compact Ion Meter C-141 NO_3^- (Spectrum™ Technologies, Inc., Aurora, IL).

Yield data were collected by picking fruit weekly if at the blush or a later stage of ripening 7 and 5 times in 2013 and 2014, respectively. The fruit was counted, weighed, and sorted for marketability. Weight and number of total and marketable fruit were recorded. Average marketable fruit weight was calculated as marketable fruit weight/marketable fruit number. Marketable yield percentage was calculated as marketable fruit weight/total fruit weight×100%.

Fruit quality including °Brix, pH and TA was measured using three to four representative fruit in similar ripening stages for each subplot from the 3rd and 5th harvests in 2013 and from the

3rd, 4th and 5th harvests in 2014. The fruit was washed, cut into quarters without the core, combined a quarter from each fruit, and blended into homogenized puree samples which were stored at -20 °C until analysis. Two lab replicates of the composite sample for each subplot were analyzed in both years. Homogenate was filtered through a Kimwipe. °Brix was measured using a digital refractometer (American Optical 10480 Mark II; Reichert, Inc., Depew, NY) as described by Kleinhenz and Bumgarner (2013b). An aliquot of the puree (40 mL) was diluted with 10 mL deionized water and used to quantify pH with a Fisher Scientific Accument AB15 Basic Meter (Fisher Scientific, Pittsburgh, PA). The diluted homogenate was also used to calculate TA by titrating the puree with 0.1 N NaOH to pH 8.2.

Data from the 2013 and 2014 experiment were analyzed separately by the GLIMMIX program in SAS 9.4 (SAS Institute, Cary, NC). All data were analyzed as a split-plot design with fertilization, rootstock, fertilization-rootstock interaction as the fixed factors, block and block-fertilization interaction as the random factors, except for the first vegetative harvest data, truss number, and duration of plant growth and fruit development, where fertigation treatments were not applied or were just applied before these data were collected and they were analyzed as a completely randomized block design with rootstock as the fixed factor and block as the random factor. For all data, mean separations were conducted by the LSMEANS statement with LINES and BYLEVEL options in the GLIMMIX program.

Results

Plant growth showed inconsistent trends between 2013 and 2014. Truss numbers per plant were smaller in ungrafted control versus the three grafted combinations 4, 6 and 8 weeks after grafting in 2013, but the opposite was true in 2014 (Figure 8). Similar trends were found in the first vegetative harvest. Ungrafted plants had smaller aboveground, stem, and leaf fresh and dry weight, as well as leaf area compared to grafted plants in 2013 (Table 20). However, ungrafted plants had larger aboveground, stem, and leaf fresh and dry weight compared to grafted

plants in 2014 (Table 21). Grafted plants with ‘Maxifort’ as the rootstock had numerically or significantly smaller plant growth compared to those with ‘Emperador’ and ‘320’ as rootstocks in the first vegetative harvest in both years. Ratios of stem fresh weight to aboveground fresh weight and stem dry weight to aboveground dry weight did not differ in 2013, but they were smaller in grafted plants with ‘Maxifort’ as the rootstock in 2014. Duration from transplanting to anthesis of the first flower was longer in ungrafted plants versus grafted ones in both years (Figure 9). Duration from anthesis of the first flower to appearance of the first green fruit larger than 5 mm in diameter was shorter in ungrafted plants versus grafted ones in 2013, but not different among rootstock treatments in 2014. Duration of fruit ripening was not different among rootstock treatments in 2013, but shorter in ungrafted plants and grafted plants with ‘Maxifort’ as the rootstock in 2014. In the second vegetative harvest, ungrafted plants had smaller aboveground, stem, and leaf fresh and dry weight, leaf area and leaf NO_3-N content than the grafted plants in 2013 (Table 22). There was no difference among the three grafted combinations. However, in 2014, there was no difference in plant growth between grafted and ungrafted plants, except for stem dry weight which was larger in ungrafted plants (Table 23). Plant growth and leaf NO_3-N content were significantly or numerically larger in the per-plant fertilization plus standard fertigation treatment than those in the pre-plant fertilization only treatment in both years. There were no interactions between fertilization and rootstock treatments in either year.

Fruit yield was not affected by fertilization treatment in 2013, but marketable fruit weight and number were larger under the pre-plant fertilization plus fertigation treatment than those under the pre-plant fertilization only treatment in 2014 (Tables 24-25). Total and marketable fruit number and marketability percentage were larger in grafted plants compared to their ungrafted counterparts in 2013. Yield was not significantly but numerically larger in grafted plants versus ungrafted ones in 2014. There were no significant interactions between fertilization and rootstock treatments in either year.

Fruit quality showed inconsistent trends in 2013 and 2014 (Tables 26-27). In 2013, °Brix, pH, and TA were not affected by fertilization treatments. °Brix was larger in ungrafted plants and grafted plants with '320' as the rootstock than the other two grafted combinations. PH was smaller while TA was larger in grafted plants with 'Emperador' as the rootstock than the other three rootstock treatments. In 2014, pH and TA were larger under the pre-plant fertilization plus fertigation treatment. °Brix was larger in ungrafted plants than the grafted combinations. PH was larger in grafted plants with 'Emperador' as the rootstock. TA was not different among rootstock treatments.

Discussion

Results demonstrated that 1) the pre-plant fertilization plus standard fertigation treatment increased plant growth in both years and enhanced yield in 2014 compared to the pre-plant fertilization only treatment; 2) there were no significant interactions between fertilization and rootstock treatments in most of the measured variables; 3) regardless of the different trends in plant growth between two years, grafted plants tended to have a higher yield than the ungrafted control; 4) °Brix tended to be higher in fruits from ungrafted plants versus grafted ones, while pH and TA had inconsistent trends among fertilization and rootstock treatments.

The higher rate of fertilization increased plant growth and yield in both grafted and ungrafted plants, without interactions with rootstock treatments. In 2013, yield was not influenced by fertilization treatments, which may be due to adequate background nutrients available to plants without fertigation.

Grafted plants have a higher yield potential than the ungrafted ones, regardless of fertilization treatments and different trends of plant growth. The different trends between grafted and ungrafted plants in plant growth across the 2 years may be due to the different plant ages. The relative age of ungrafted plants compared to grafted ones was 13 d younger in 2014 than that in 2013. In both years, ungrafted plants required a longer duration from transplanting to flowering

than the grafted ones, which may be also caused by the relatively younger ages of ungrafted plants than the grafted ones. In future research when both grafted and ungrafted plants are included, their relative ages should be standardized. The trends in yield and plant growth seemed to be related. In 2013, both plant growth and yield were significantly larger in grafted plants versus ungrafted ones; in 2014, plant growth was not different or smaller in grafted plants versus ungrafted ones and yield was numerically but not significantly larger in grafted plants. Regardless, yield tended to be higher in grafted plants, under both high and low fertilization treatments and when plant growth was higher, not different and lower in grafted plants. This confirms the potential of using grafting to improve the productivity of tomato. Consistent with previous studies (Djidonou et al., 2013, 2015), fertilizer requirement is lower for grafted plants than that for ungrafted plants to achieve the same yield goal of the tested combinations in Florida. In the study, the growing conditions were free of significant biotic or abiotic stresses. It is worth testing the effects of grafting extensively under different growing conditions.

Fruit quality was inconsistently affected by fertilization treatments in this study. Bénard et al., (2009) reported that lower nitrogen supply reduced yield and plant vegetative growth while improved fruit quality by lowering acid and increasing soluble sugar content. In this study, the effects of reduced nitrogen fertilization on yield and plant growth were consistent with the previous report, but the trends in fruit quality were different. The pre-plant fertilization only treatment resulted in lower pH and TA values, which may indicate certain compounds as buffer compositions in tomato fruit were influenced by the fertilization treatments (Paulson and Stevens, 1974). °Brix as an indicator of sugar content did not differ between fertilization treatments. This study lowered other nutrient supply along with nitrogen while the previous report only lowered nitrogen supply, which might be responsible for the different results. Fruit quality variables may correlate with yield (Ben-Oliel et al., 2005). Neither yield nor fruit quality was affected by fertilization treatments in 2013, whereas, both yield and fruit quality were affected by fertilization treatments in 2014.

Trends of fruit quality including °Brix, pH and TA were also inconsistent among the grafted combinations and the ungrafted control. The effects of grafting on fruit quality are not conclusive and depend on production environments, harvest dates, and rootstock-scion combinations (Davis et al., 2008; Rouphael et al., 2010). Several previous studies reported no difference in fruit quality including soluble solids content, sugars, pH or TA between grafted and ungrafted tomatoes (Barrett et al., 2012; Gioia et al., 2010; Matsuzoe et al., 1996; Savvas et a., 2011). By contrary, Khah et al. (2006) reported that °Brix and pH were not affected by grafting and acidity was higher in a grafted combination compared to the ungrafted control in the open field tomato production. Flores et al. (2010) found that °Brix and TA increased in specific grafted combinations of tomato. Grafting reduced concentration of soluble solids, increased TA, and did not affect pH in tomato production in greenhouses (Turhan et al., 2011). Soluble solids and organic acids were lower in grafted plants versus ungrafted ones (Pogonyi et al., 2005). In this study, grafted combinations with 'Maxifort' and 'Emperador' as rootstocks resulted in lower °Brix values. Trends of pH and TA among rootstock treatments were inconsistent between the two years, which may be caused by different growing conditions across years.

To summarize, plant growth, yield, and fruit quality were influenced by fertilization regimens and grafting. Grafted plants had a higher yield potential than ungrafted ones under both high and low rates of fertilization treatments in conventional, open field fresh market tomato production. However, fruit quality needs to be carefully monitored since grafted plants resulted in lower °Brix values compared to the ungrafted control in this study.

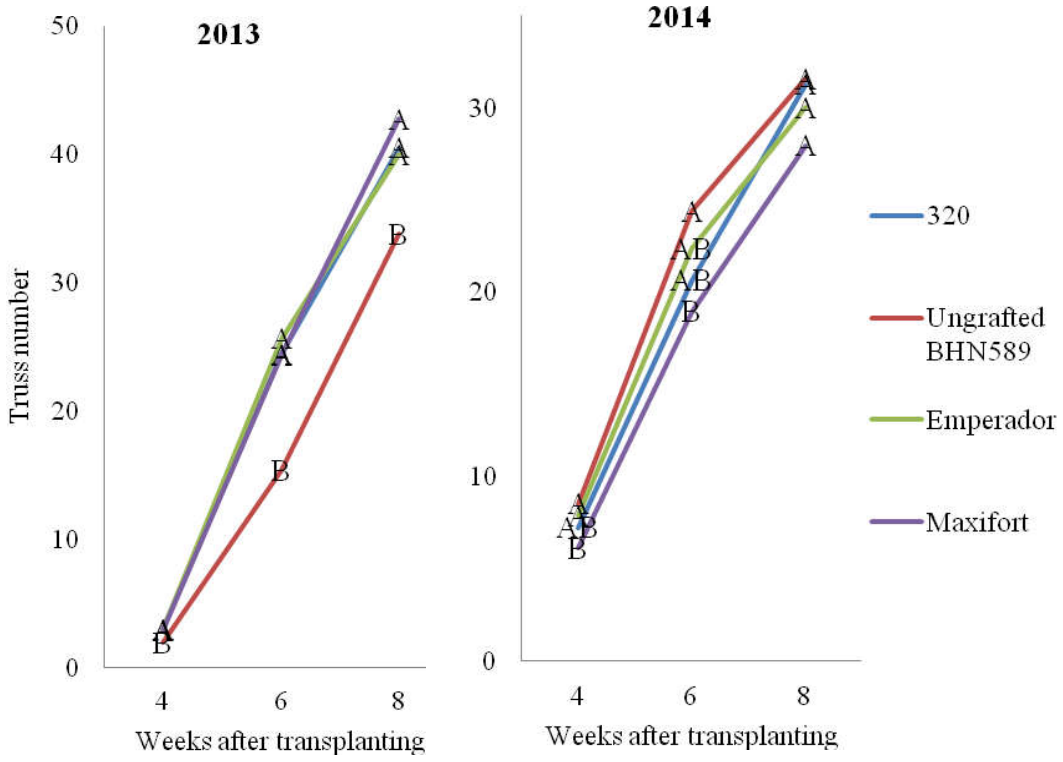

Figure 8. Truss number per plant 4, 6, 8 weeks after transplanting affected by rootstock treatments. Means in the same week followed by the same letter are not significantly different ($P < 0.05$)

Table 20. Effects of rootstock treatments on tomato plant growth of the first vegetative harvest in 2013 in Wooster, OH

Rootstock	Aboveground fresh wt (g)	Stem fresh wt (g)	Leaf fresh wt (g)	Aboveground dry wt (g)	Stem dry wt (g)	Leaf dry wt (g)	Leaf area (cm^2)	Stem to aboveground fresh wt (%)	Stem to aboveground dry wt (%)
Maxifort	465b[z]	141b	325a	48b	11b	37b	4410b	30a	24ab
Emperador	538a	165a	373a	55ab	13a	42ab	5353a	31a	24a
320	519ab	155ab	365a	57a	13ab	44a	5040ab	30a	23ab
Ungrafted BHN 589	247c	75c	172b	28c	6c	22c	2474c	30a	21b
P values	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.9	0.2

[z] Means within a column followed by the same letter are not significantly different ($P < 0.05$).

Table 21. Effects of rootstock treatments on tomato plant growth of the first vegetative harvest in 2014 in Wooster, OH

Rootstock	Aboveground fresh wt (g)	Stem fresh wt (g)	Leaf fresh wt (g)	Aboveground dry wt (g)	Stem dry wt (g)	Leaf dry wt (g)	Leaf area (cm^2)	Stem to aboveground fresh wt (%)	Stem to aboveground dry wt (%)
Maxifort	847c[z]	229c	618c	86c	17c	69b	9639a	27c	19b
Emperador	1045ab	304b	741ab	98b	22b	77b	10468a	29b	22a
320	1000b	300b	700b	101b	23b	78b	9960a	30ab	23a
Ungrafted BHN 589	1160a	359a	801a	119a	27a	91a	11282a	31a	23a
P values	<.0001	<.0001	<.0001	<.0001	<.0001	0.0003	0.3	<.0001	0.0001

[z] Means within a column followed by the same letter are not significantly different ($P < 0.05$).

Table 22. Effects of fertilization and rootstock treatments on tomato plant growth of the second vegetative harvest in 2013 in Wooster, OH

Factors	Aboveground fresh wt (g)	Stem fresh wt (g)	Leaf fresh wt (g)	Aboveground dry wt (g)	Stem dry wt (g)	Leaf dry wt (g)	Leaf area (cm^2)	Leaf NO_3-N (ppm)
Fertilization								
Pre-plant fertilization + fertigation	3804a[z]	1496a	2308a	414a	105a	309a	30639a	250a
Pre-plant fertilization only	3351b	1322a	2029b	371a	94a	277a	25393a	228a
Rootstock								
Maxifort	3986a	1573a	2412a	420a	110a	310a	31018a	277a
Emperador	3715a	1488a	2228a	404a	101a	303a	28611a	289a
320	3751a	1440a	2311a	420a	109a	311a	29548a	229ab
Ungrafted BHN 589	2859b	1136b	1723b	327b	79b	248b	22886b	161b
P values								
Fertilization	0.04	0.07	0.04	0.07	0.1	0.09	0.06	0.8
Rootstock	<.0001	<.0001	<.0001	<.0001	0.0004	0.0007	0.002	0.05
Rootstock × fertilization	0.4	0.06	0.7	0.2	0.2	0.3	0.4	0.8

[z] Means within a factor category and a column followed by the same letter are not significantly different ($P < 0.05$).

Table 23. Effects of fertilization and rootstock treatments on tomato plant growth of the second vegetative harvest in 2014 in Wooster, OH

Factors	Aboveground fresh wt (g)	Stem fresh wt (g)	Leaf fresh wt (g)	Aboveground dry wt (g)	Stem dry wt (g)	Leaf dry wt (g)	Leaf area (cm^2)	Leaf NO_3-N (ppm)
Fertilization								
Pre-plant fertilization + fertigation	3111a[z]	948a	2163a	329a	97a	232a	27327a	255a
Pre-plant fertilization only	2456b	818a	1636b	264b	87a	177b	20178b	204a
Rootstock								
Maxifort	2742a	873a	1868a	282a	86b	196a	23712a	204a
Emperador	2846a	879a	1967a	294a	88b	206a	24798a	279a
320	2764a	864a	1900a	300a	90b	210a	24242a	207a
Ungrafted BHN 589	2795a	919a	1874a	311a	104a	206a	22405a	228a
P values								
Fertilization	0.05	0.2	0.03	0.01	0.08	0.009	0.03	0.3
Rootstock	0.9	0.7	0.7	0.3	0.003	0.6	0.5	0.4
Rootstock × fertilization	0.5	0.3	0.6	0.5	0.07	0.7	0.6	0.7

[z] Means within a factor category and a column followed by the same letter are not significantly different ($P < 0.05$).

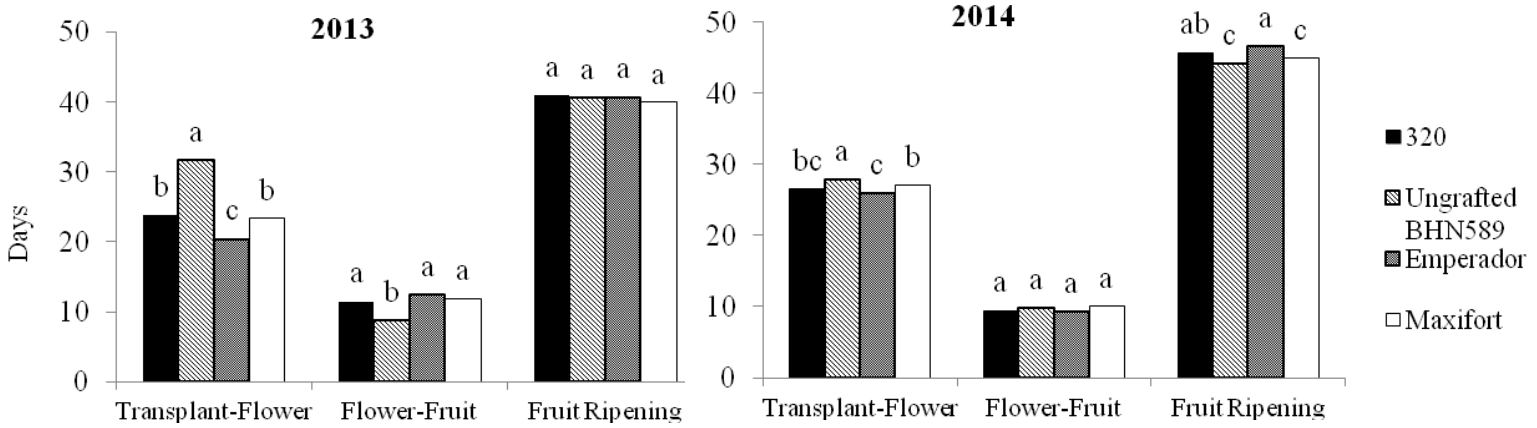

Figure 9. Duration of plant growth and fruit development affected by rootstock treatments. Means within a category followed by the same letter are not significantly different ($P < 0.05$). Transplant-Flower (from transplanting to anthesis of the first flower); Flower-Fruit (from anthesis of the first flower to appearance of the first green fruit larger than 5 mm in diameter); Fruit Ripening (from appearance of the first green fruit larger than 5 mm in diameter to appearance of the first red fruit reaching the blush stage)

Table 24. Effects of fertilization and rootstock treatments on tomato yield in 2013 in Wooster, OH

Factors	Total fruit wt per plant (kg)	Total fruit number per plant	Marketable fruit wt per plant (kg)	Marketable fruit number per plant	Average marketable fruit wt (g)	Marketability percentage (%)
Fertilization						
Pre-plant fertilization + fertigation	10.2a[z]	43a	4a	19a	242a	44a
Pre-plant fertilization only	10.9a	44a	5a	21a	262a	49a
Rootstock						
Maxifort	10.9ab	47a	5a	22a	248ab	49a
Emperador	10.2ab	44a	5ab	20ab	242b	45ab
320	11.4a	46a	6a	24a	251ab	52a
Ungrafted BHN 589	9.7b	36b	4b	15b	269a	39b
P values						
Fertilization	0.4	0.8	0.2	0.5	0.1	0.3
Rootstock	0.2	0.01	0.1	0.01	0.2	0.01
Rootstock × fertilization	0.1	0.1	0.7	0.5	0.6	0.9

[z] Means within a factor category and a column followed by the same letter are not significantly different ($P < 0.05$).

Table 25. Effects of fertilization and rootstock treatments on tomato yield in 2014 in Wooster, OH

Factors	Total fruit wt per plant (kg)	Total fruit number per plant	Marketable fruit wt per plant (kg)	Marketable fruit number per plant	Average marketable fruit wt (g)	Marketability percentage (%)
Fertilization						
Pre-plant fertilization + fertigation	11a[z]	46a	9a	37a	244a	79a
Pre-plant fertilization only	10a	41a	7b	30b	245a	73a
Rootstock						
Maxifort	10ab	41b	8ab	33ab	252a	78a
Emperador	12a	50a	9a	37a	245ab	74a
320	10b	43ab	8ab	33ab	237b	77a
Ungrafted BHN 589	9b	39b	7b	29b	244ab	74a
P values						
Fertilization	0.1	0.1	0.05	0.04	0.9	0.08
Rootstock	0.05	0.08	0.08	0.09	0.1	0.5
Rootstock × fertilization	0.9	0.7	0.7	0.5	0.7	0.3

[z] Means within a factor category and a column followed by the same letter are not significantly different ($P < 0.05$).

Table 26. Effects of fertilization and rootstock treatments on tomato fruit quality in 2013 in Wooster, OH

Factors	°Brix	pH	TA
Fertilization			
Pre-plant fertilization + fertigation	4.5a[z]	4.2a	7.5a
Pre-plant fertilization only	4.5a	4.2a	7.4a
Rootstock			
Maxifort	4.4bc	4.24a	7.3bc
Emperador	4.3c	4.19b	8.0a
320	4.7a	4.24a	7.1c
Ungrafted BHN 589	4.6ab	4.23a	7.6ab
P values			
Fertilization	0.9	0.6	0.8
Rootstock	0.01	0.006	0.0004
Rootstock × fertilization	0.3	0.9	0.05

[z] Means within a factor category and a column followed by the same letter are not significantly different ($P < 0.05$).

Table 27. Effects of fertilization and rootstock treatments on tomato fruit quality in 2014 in Wooster, OH

Factors	°Brix	pH	TA
Fertilization			
Pre-plant fertilization + fertigation	4.4a[z]	4.22a	7.4a
Pre-plant fertilization only	4.6a	4.17b	6.6b
Rootstock			
Maxifort	4.3c	4.20ab	7.0a
Emperador	4.1d	4.22a	6.9a
320	4.7b	4.18b	7.1a
Ungrafted BHN 589	4.9a	4.18b	6.9a
P values			
Fertilization	0.06	0.01	0.01
Rootstock	<.0001	0.02	0.4
Rootstock × fertilization	0.2	0.6	0.02

[z] Means within a factor category and a column followed by the same letter are not significantly different ($P < 0.05$).

Chapter 7: Conclusion

Vegetable grafting is a novel application of an old practice. It can immediately combine the desirable traits of two plants into one physical hybrid. Grafting has the potential to improve tomato productivity, by enhancing abiotic and biotic stress tolerance and resource-use efficiency. However, the new application of grafting to tomato production raises new questions and requires research-based information on this technology. The goal of this program was to improve grafting technologies, including to enhance the efficiency of grafted tomato seedling propagation and to maximize the benefits of using grafted plants for tomato production.

In total, seven studies were completed over the past 4 years at the OARDC in Wooster, OH and on thirty-one farms of grower-cooperators. These studies addressed challenges across three stages throughout the process of using grafting for tomato production: 1) seedling preparation before grafting, 2) healing of grafted seedlings immediately after grafting, and 3) grafted plant production in the open field. Major findings and future perspectives include:

1) Tools such as seedling vigor indexes are useful for documenting rootstock and scion traits and scheduling seedling preparation for grafting; future study can further develop and standardize these tools.

With the increasing interest in tomato grafting, the number of commercial rootstock cultivars increased to be 60 in 2016. However, very limited information on the traits of these rootstocks is available. Seedling growth vigor especially relative to scion cultivars is an important but undocumented rootstock trait. Seedling vigor can be an indicator of the vigor of grafted plant

growth and fruiting, which can guide the cultivar selection based on production needs. The information on relative seedling growth vigor of rootstock and scion cultivars can guide the scheduling of seeding to ensure that rootstock and scion seedlings reach the suitable size for grafting at the same time. Therefore, a standardized procedure for evaluating seedling growth vigor is useful for grafting operations. The method developed in this program included plant growth and environment conditions in the calculation of seedling growth vigor. It has provided a novel and solid concept which can be further improved, e.g. to achieve comparable vigor values under different conditions for a specific cultivar, so that the vigor indexes can predict a rootstock performance across growing conditions. With the further development, rootstock developers can provide information on vigor using the seedling vigor indexes as a standardized procedure.

2) The current practice of healing grafted seedlings should be reconsidered; future study may determine the optimal humidity conditions and test potential healing chamber designs based on the better knowledge of light and temperature effects on healing.

The promoted resumption of plant growth by exposing grafted plants to high light (up to 300 μmol/m^2/s) under moderate temperature (25°C during the lid period) questioned the current practice of healing grafted tomato seedlings under dim or dark conditions. The main reason for reducing the light intensity in the current practice is to avoid excessive heat built up inside the healing chamber. If alternative practices such as using LED light and controlled environment are feasible to decouple light and temperature control, increased light intensity may benefit graft healing. However, in healing chambers where temperature control is not reliable, reducing light intensity may be necessary since the higher temperature at 35 °C during the lid period under 300 μmol/m^2/s can reduce survival. Future study should also test the effects of humidity on the healing of grafted tomato seedlings, and the interactions among light, temperature and humidity. These results can provide guidance for the designs of healing chambers in the future. Different facilities such as movable chambers with high environment controls and chambers constructed

indoors or outdoors with low environment controls should be designed and tested to create appropriate conditions for graft healing and meet different needs.

3) Grafted plant performance is affected by growing conditions and management, which needs to be extensively studied in the future, possibly connecting with the seedling vigor traits of rootstocks and scions.

In this program, no obvious biotic or abiotic stresses were present and two fertilization regimens were tested. Grafted plants had a higher yield potential than the ungrafted ones, regardless of the different plant growth and different fertilization regimens. Therefore, grafting can be an effective tool for improving tomato productivity. However, °Brix values of fruit from grafted plants were lower than those from ungrafted controls, while the trends of pH and TA were inconsistent. The effects of grafting on fruit quality were inconclusive and require further investigation and careful monitoring when grafting is adopted. Plant performance of grafted tomato differed in previous reports and across the two years in this program, which suggested that grafted plant performance is affected by growing conditions and management as well as grafted combinations. Extensive tests of grafted plant performance under different situations should be continued in order to improve the understanding of how grafting and management interacts and how to maximize the benefits of using grafted plants. Future studies may also test the correlation between the seedling vigor values of rootstocks and scions and the field performance of their grafted plants.

Appendix A: Midwest Vegetable Trial Report for 2014 Eighteen Rootstock and Five Scion Tomato Varieties: Seedling Growth Rates Before Grafting and Success in Grafting the Ninety Variety Combinations (Ohio)

Introduction

The use of grafted plants to lessen the impacts of abiotic and biotic stresses is increasing in the U.S. open field- and high tunnel-based production. Growers can purchase or prepare their own grafted plants. Regardless, all who prepare grafted plants benefit from research-based information regarding the compatibility of various rootstock-scion combinations and the growth of rootstock and scion seedlings before grafting. Rootstock and scion seedlings can be grafted only when their stem diameters are similar and approximately 1.5 - 3.0 mm. Therefore, the relative growth rate of rootstock and scion seedlings strongly influences sowing and grafting schedules. Genetically or physically mismatched rootstock and scion seedlings can have significant negative consequences. To our knowledge, the number of commercially available rootstock varieties has increased nearly 10-fold in approximately six years but information regarding rootstock compatibility and seedling vigor is largely unavailable. We documented the growth rate of eighteen tomato rootstock and five scion varieties and tested the graft survivorship and performance of ninety potential rootstock-scion combinations representing growers' production needs and goals.

Materials and Methods

Plant materials and seeding conditions

Eighteen tomato commercial rootstock and five scion varieties were selected based on grower input and our familiarity with varieties. Tomato growers throughout the Midwest and North-Central U.S. were contacted directly through the collaboration and use of 19 organic certifying agencies, 25 grower associations, 5 trade publications, 4 listservs, and 11 farmer groups. Information, particularly about disease resistance, of commercial tomato rootstocks was obtained from seed catalogs and seed company websites. Grower input was used in cultivar selection. Collectively, the rootstock varieties were developed by 12 companies and contain approximately 11 disease tolerance/resistance packages. The selected scion varieties represent hybrid and heirloom and round- and oblong-fruited types. A list of the rootstock varieties included in the study and their characteristics are shown in Table 28 and in a reference table updated annually (http://www.vegetablegrafting.org/wp/wp-content/uploads/2012/08/usda-scri-etal-tomato-rootstock-table-feb2013-mk-1.pdf).

Two rounds of growth and compatibility evaluations were completed February-April, 2014 in a climate-controlled greenhouse located at the Ohio Agricultural Research and Development Center in Wooster, OH. In the greenhouse, the average temperature was 74 °F, relative humidity was 37%, and photosynthetically active radiation (PAR) was 130 µmol $m^{-2}s^{-1}$ during round 1; the average temperature was 75 °F, relative humidity was 46%, and PAR was 197 µmol $m^{-2}s^{-1}$ during round 2. Seed was sown on 25, 27 and 28 Feb. and 1 Mar. 2014 for round 1, and on 26 and 28 Mar. and 2 Apr. 2014 for round 2. All seed were sown into 96-cell trays filled with growing medium (PRO-MIX® MP MYCORRHIZAE™ Organik™; Premier Tech, Canada). Trays were placed on a capillary mat on a bench with an automatic irrigation system.

Stem diameter growth measurement

Stem diameter was measured 1 cm below the cotyledons 12, 15, and 18 d after sowing. One more measurement was taken 26 d after sowing on the three slowest-growing varieties (Trooper, Estamino, and RST-04-105) in round 1. The data were fit to a linear model using Proc Reg in SAS (version 9.3; SAS Institute, Cary, NC). R-square of each fitting was from 0.47 to 0.90 in round 1 and 0.43 to 0.88 in round 2. Estimated parameters in the linear model were used to calculate predicted days needed to reach 1.5 mm and 3.0 mm (the minimum and maximum stem diameter suitable for grafting) in Microsoft Excel (2010). The days to reach 3.0 mm were out of the range of actual measurement; therefore, they are projections.

Grafting procedure and healing conditions

The day of emergence was noted for each plant. Plants were grafted when they reached 1.5 to 2.5 mm in stem diameter. Plants of each cultivar that emerged within the same 3-d period and that were similar in size were selected in order to minimize within-cultivar plant to plant variation. In round 1, grafting days were March 19, 21, 24, and 25, 2014; in round 2, grafting days were April 12, 14, 15, 16, 18, and 21, 2014. The cleft graft method (http://hcs.osu.edu/vpslab/grafting-guide) was used to graft all plants. Rootstock and scion seedlings at similar growth stage and with matching stem diameters were selected.

Immediately after grafting, plants were placed in a healing chamber in the same greenhouse room used for seedling production for two weeks until evaluation. The healing chamber was constructed and used as described previously (http://hcs.osu.edu/vpslab/grafting-guide) using a polyvinyl chloride (PVC) frame covered by one layer of clear plastic sheeting overlain by one layer of shade cloth (47% light transmission in PAR). Within the chamber, an automatic irrigation system with drippers and foggers was used to maintain high moisture. The four sides of the healing chamber were opened gradually over time, as weather and plant status allowed, to lower relative humidity and limit adventitious root development. Temperature and

relative humidity in the healing chamber were recorded continuously at 5-min intervals with Hobo ProV2 data loggers (version 2.5.0, Onset Computer Co., Pocasset, MA, USA) throughout the study. The average temperature in the healing chamber during round 1 was 73 °F and relative humidity was 87%; during round 2, the average temperature and relative humidity was 74°F and 88%, respectively.

Graft survivorship assessment

Two weeks after grafting, graft survival was rated based on scion appearance using metrics as described previously (http://hcs.osu.edu/vpslab/grafting-guide; Johnson and Miles, 2011). Grafted plants with completely wilted scions were regarded as dead while all other plants were scored as living. The number of living plants was counted for each combination, and survivorship was calculated as the number of living plants divided by the total number of plants grafted for each combination.

Survivorship data were collected for ninety rootstock-scion combinations. Due to the limited grafting capacity of a grafter on a day, the experiment was conducted using an augmented design. Ninety rootstock-scion combinations were assigned to grafters randomly, and grafted on different days as soon as plants grew to 1.5-2.5 mm in stem diameter. Each grafter and graft day combination was treated as a block. Self-grafted Cherokee Purple was used as the common control and repeated twice within each block at random. Twelve grafted plants were grafted for each combination. The experiment was repeated twice in February-March and March-April, 2014, within the months allotted for commercial propagation of grafted tomato seedlings.

Data analysis was conducted by the Proc Glimmix procedure in SAS (version 9.3; SAS Institute, Cary, NC). Survivorship was the response variable, and rootstock, scion, rootstock*scion interaction and block were treated as fixed effects. Treatment means were separated using a pdiff option in the LSMEANS statement at $\alpha = 0.05$. The Tukey method was applied for multiple comparison adjustments to analyze the differences in least square means.

On-farm evaluation of grafted plants

Growers were invited to nominate their farm as a site for the summertime evaluation of grafted plants prepared in phase 1 of the study. Invitations to self-nominate their farm were issued to growers through the collaboration and use of 24 organic certifying agencies, 13 grower associations, representatives of 6 industry trade publications, 5 seed companies and 4 grower-oriented listservs operating in the Midwest and North Central U.S. Requests to participate in the evaluation exceeded the number of plants available for distribution by three-fold. A total of 86 growers submitted requests through an online form (www.hcs.osu.edu/vpslab). Growers were selected on a first come, first serve basis by order of receipt of request and then by state the grower was located in. A total of approximately 1,000 grafted plants representing 90 rootstock-scion combinations were shipped in mid-April, 2014 to 31 growers in 13 states for on-farm evaluation.

Results and Discussion

Predicted days needed for each cultivar to grow to 1.5 mm and 3.0 mm in stem diameter varied among varieties (Table 29). In round 1, Arnold and Trooper needed 16 and 30 d to reach 1.5 mm, the shortest and longest times among tested varieties, respectively. Most varieties needed 17-21 d to reach 1.5 mm stem diameter, although RST-04-105 and Estamino were found to require longer, 23 and 24 d respectively. In round 2, Kaiser and Trooper required 10 and 19 d to reach 1.5 mm diameter (the shortest and longest among all varieties, respectively) while most varieties required 12-15 d to reach 1.5 mm stem diameter. The five scions required an intermediate amount of time to reach 1.5 mm stem diameter (19 or 20 d in round 1 and 13 or 14 d in round 2), although San Marzano 2 was estimated to require 17 and 12 d in rounds 1 and 2, respectively. The amount of time that varieties were found or projected to attain stem diameters

of 1.5 m – 3.0 mm (the grafting window) ranged from 10 to 25 d in round 1 and 6 to 16 d in round 2.

Survivorship in either round of evaluation did not differ significantly among the ninety rootstock-scion combinations tested here (Table 30). Graft survival exceeded 92% in all combinations (Table 31) with a study-wide average of 97%. However, survivorship differed by day of grafting and grafter in round 2 ($P = 0.02$).

Growers continue to provide quantitative and qualitative information from on-farm evaluations of grafted plants provided to them. The information will be submitted for inclusion in the 2015 Midwest Vegetable Variety Trial Report. A preliminary assessment of information available to date suggests that grafted plant performance varied by rootstock-scion combination and farm and that growers remain interested in additional evaluations and data on grafted plant performance.

We documented the seedling growth rate of eighteen commercial rootstock and five scion varieties and the percent survivorship of ninety grafted combinations. We also tracked graft survivorship by grafter and day of grafting and provided grafted plants to thirty-one growers in thirteen states for evaluation. We learned that: a) growth rates, including stem diameters which are important in grafting, vary significantly among rootstock varieties, b) that the growth rates of many rootstock varieties may differ from scion varieties, c) that graft survivorship may differ less than growth rates, d) that grafter and grafting-day conditions may influence survivorship, e) that on-farm performance of grafted plants is likely to differ among rootstock-scion combinations and locations, and f) that growers remain strongly interested in grafted plants as production tools. Based on these findings, genetic incompatibility may be less of a concern than scheduling rootstock and scion sowing and grafting periods, identifying skilled grafters and optimizing the condition of grafting stock and grafting-healing room conditions.

Table 28. List of eighteen commercial tomato rootstock and five scion varieties used in this study

Rootstock Variety	Seed company/ distributor	Rootstock Variety	Seed company/ distributor	Scion Variety	Seed company/ distributor
Aiboh	Asahi Industries	Kaiser	Rijk Zwaan	Brandywine	NE Seed
Akaoni	Asahi Industries	Maxifort	DeRuiter Seeds	Better Boy	NE Seed
Aooni	Asahi Industries	Resistar	Hazera Seeds	Celebrity	NE Seed
Armada	Takii Seed	RST-04-105	DP Seeds	Cherokee Purple	NE Seed
Arnold	Siegers Seed Co.	RST-04-106	DP Seeds	San Marzano 2	NE Seed
B.B.	Takii Seed	Shield	Rijk Zwaan		
Beaufort	DeRuiter Seeds	Stallone	Rijk Zwaan		
Cheong Gang	Seminis Vegetable	Supernatural	A.P. Whaley Seeds		
Estamino	Enza Zaden	Trooper	Seedway		

Note 1. None of the seed used in this study was treated.
Note 2. Seed of only Kaiser and Stallone was pelleted; all other seed was not pelleted.
Note 3. Seed of only Arnold, Beaufort, Kaiser, Maxifort, Shield, and Stallone was primed; all other seed was not primed.

Table 29. Predicted days after sowing to reach 1.5 mm and 3.0 mm stem diameter (the range over which plants can be grafted reliably) of 18 tomato rootstock and 5 scion varieties. Each bar is bounded on the left and right by the days at which stem diameter is expected to be 1.5 mm and 3.0 mm, respectively. White color bars represent round 1 and grey color bars represent round 2

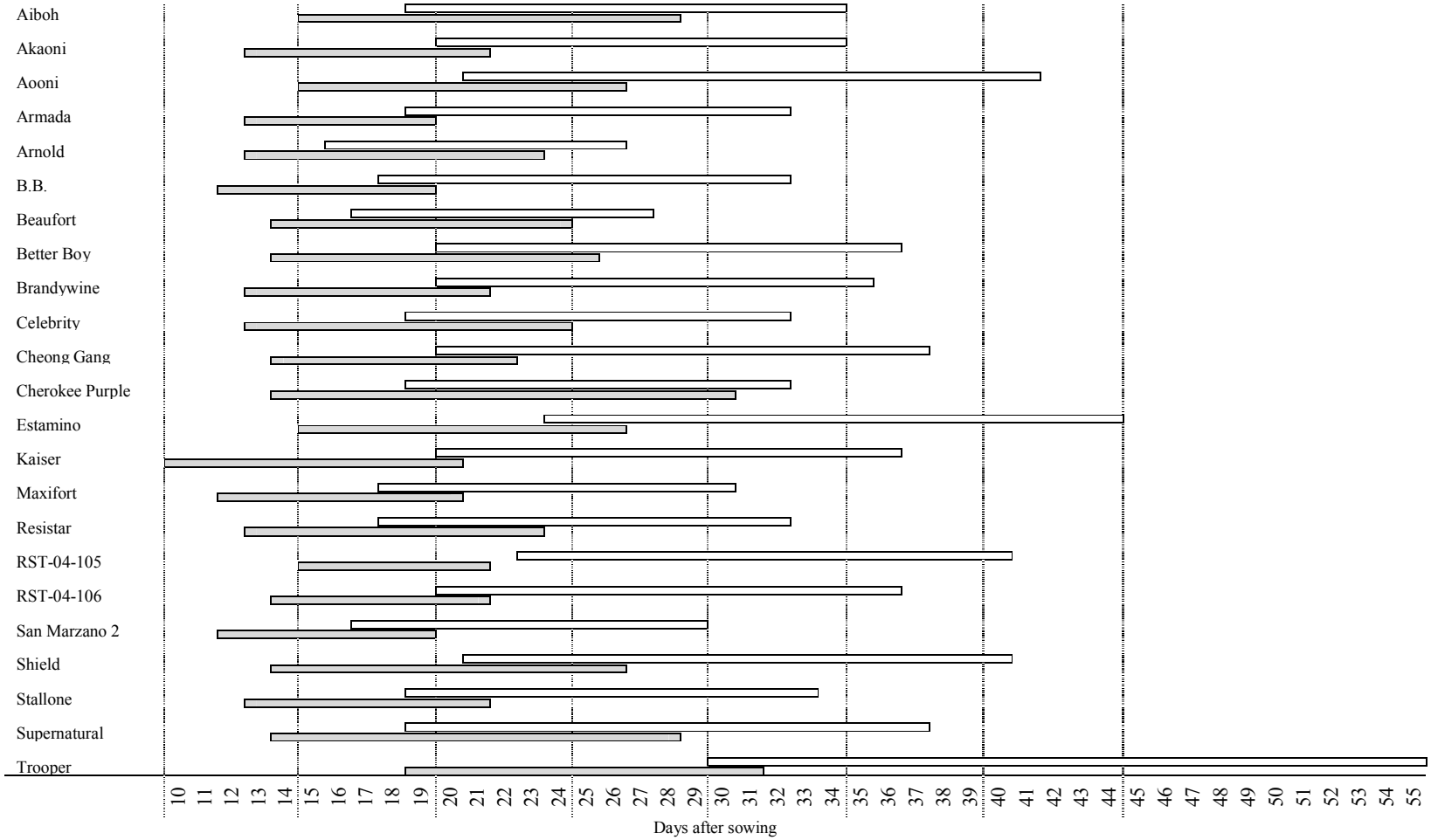

Table 30. Type III tests of fixed effects (rootstock, scion, rootstock*scion interaction, and block) on survivorship using the GLIMMIX procedure (SAS version 9.3; SAS Institute, Cary, NC)

Effect	Survivorship			
	Round 1		Round 2	
	DF	*p*	DF	*p*
Rootstock	17	0.65	17	0.52
Scion	4	0.82	4	0.97
Rootstock × scion	68	0.99	68	0.64
Block	17	0.52	14	0.02

Table 31. Graft survivorship (%) of eighteen tomato rootstocks and five scions. N = 10 for rootstock, N = 36 for scion, N = 66 for self-grafted control. Data are presented as means ± SE

Variety	Survivorship	Variety	Survivorship
Rootstock		*Scion*	
Aiboh	97 ± 1	Brandywine	97 ± 1
Akaoni	100 ± 0	Better Boy	98 ± 1
Aooni	97 ± 1	Celebrity	95 ± 1
Armada	97 ± 1	Cherokee Purple	98 ± 1
Arnold	97 ± 1	San Marzano 2	97 ± 1
B.B.	92 ± 3	Self-grafted control	95 ± 1
Beaufort	99 ± 1		
Cheong Gang	98 ± 1		
Estamino	98 ± 1		
Kaiser	96 ± 3		
Maxifort	98 ± 1		
Resistar	98 ± 1		
RST-04-105	99 ± 1		
RST-04-106	97 ± 1		
Shield	95 ± 3		
Stallone	98 ± 2		
Supernatural	95 ± 3		
Trooper	100 ± 0		
Self-grafted control	95 ± 1		

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