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Leaf removal reduces scion adventitious root formation and plant growth of grafted tomato☆

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ABSTRACT

Although grafting tomato with vigorous rootstocks provides the potential for higher yield, grower adoption of this technology has been relatively slow in the United States. One way to help facilitate this transition is to develop simple propagation techniques that yield high quality grafted transplants for small-batch propagators to graft their own plants. Formation of adventitious roots (AR) from the scion can result in poor quality plants and loss of rootstock function/benefit. In this study, a series of greenhouse experiments was performed to investigate how leaf removal (LR) during the grafting procedure affects AR formation and plant growth post-grafting. We applied three treatments, 0% LR, 50% LR, and 90% LR, to the 'BHN 589' scion and then grafted them onto 'Maxifort' rootstock. The experiment included 4 replicated blocks and was conducted in three different healing chambers. Our results indicate that both 50% and 90% LR significantly decreased AR formation in the low (68% RH) humidity chamber, but only 90% LR reduced AR formation in the chambers with high (95% RH) humidity ($P < 0.05$). Using a second experimental design, we measured plant growth (height, leaf area, shoot and root biomass, stem diameter, and incidence of flowers) 24 to 52 days post-grafting to understand how leaf removal affects transplant quality (as defined by Vu et al., 2013), growth, and development. Plants with 90% LR had significantly lower leaf area and shoot biomass at day 24, but by day 52, only had reduced stem diameter and height compared to 0% LR. Leaf removal during grafting may be a viable method for propagating high quality, grafted transplants and our report indicates that the desired product (plant for sale vs. plant for use) could dictate the use of 50% vs. 90% LR as the higher leaf removal level reduced transplant quality at the estimated time of sale, but did not affect subsequent plant growth.

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1. Introduction

Grafted Solanaceae and Cucurbitaceae vegetable crops are used worldwide for managing abiotic and biotic stresses, particularly in intensively-cultivated production systems such as: greenhouses, high tunnels, small farms, and urban agricultural settings (Guan et al., 2012; Kubota et al., 2008; Lee, 1994; Louws et al., 2010). Many vegetable farmers in the United States are interested in using grafted vegetable plants for commercial production, but have limited purchasing options or ability to graft their own plants (Kubota et al., 2008; Rivard et al., 2010b). We surveyed fruit and vegetable growers (n = 265) at the 2014 Great Plain Growers Conference, in St. Joseph, MO and 19% of the respondents are using grafted vegetables, but an additional 56% are interested in either learning more or incorporating grafted plants in their production. Furthermore, 47% would prefer to graft their own transplants while 25% would rather purchase plants (C.L. Rivard, unpublished). On-farm grafted propagation (as opposed to purchasing grafted plants) is often preferred for many small-scale growers as they can match particular combinations of rootstock and scion cultivars in order to overcome site-specific issues while catering to specialty or niche markets (Rivard et al., 2010a). Tomato (*Solanum lycopersicum*) is relatively simple to graft compared to other commonly grafted vegetables such as pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.) and watermelon (*Citrullus lanstus*) (Kubota et al., 2008; Johnson et al., 2011), and is the most popular vegetable grown in high tunnels in the United States (Carey et al., 2009). Small-acreage growers, espe-

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cially in urban and peri-urban areas, typically plant small batches of various cultivars for specialty markets, which also contribute to the difficulty of purchasing grafted plants from specialized nurseries in Canada and Mexico (Kubota et al., 2008). Heirloom tomato cultivars that do not have disease resistance or production characteristics of modern hybrids can benefit from grafting. This may be why small-acreage growers are among the most interested in using grafted plants (Barrett et al., 2012; Rivard and Louws, 2008; Rivard et al., 2010a). Purchasing options for commercially grafted tomatoes in the United States is limited to a few propagation companies in the east, west, and southwest U.S. but are also available for import from Canada and Mexico. However, shipping transplants long distances causes concerns for transplant quality deterioration and possible unwanted movement of plant pathogens (Kubota et al., 2008).

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

Splice grafting, also called tube grafting, is the most commonly-used grafting method for tomato due to its relatively low cost and its effectiveness at producing high quality grafted transplants (Kubota et al., 2008; Oda, 1999; Rivard and Louws, 2011). However, because the scion is severed from its root system during the grafting procedure, post-grafting environmental management is critical so that the scion tissue stays alive while the vascular system connects to the rootstock. Therefore, grafted plants are immediately placed in an environment with high humidity and low light while the graft union heals (Bumgarner and Kleinhenz, 2014; Kubota et al., 2008; Oda, 1999; Rivard and Louws, 2011). Propagators are recommended to keep plants in the healing chamber for 7–10 days (Kubota et al., 2008; Oda, 1999; Rivard and Louws, 2011; Vu et al., 2013) while the graft union develops.

AR formation in plants is not completely understood and many exogenous factors, such as nutrition, humidity, light, temperature, and surrounding biota, in addition to endogenous factors, such as aging, phytohormones and other phytochemicals, can influence the promotion and inhibition of AR (Geiss et al., 2010). Vegetable grafting extension publications mention AR formation and speculate that it occurs due to an excessive time in the high humidity environment post-grafting (Johnson et al., 2011). Recent research reported high success rates of grafted tomato seedlings that were healed in lower humidity chambers (53%–69%) with shade cloth alone, with no plastic enclosure (Johnson and Miles, 2011; Masterson et al., 2016a). In a controlled environment study by Vu et al. (2013), optimal conditions to maximize grafted tomato plant survival rates (100%) included 90% RH in healing chambers for the first 2–3 days and 70% for the following 7–8 days. Furthermore, on day 10 post-grafting, Vu et al. (2013) compared the effect of humidity on transplant quality of grafted plants that were healed in environments with different levels of RH by comparing different parameters: percent of diseased plants, plant height, stem diameter, number of leaves, leaf chlorophyll content, leaf area, root and shoot dry biomass, shoot to root ratio, and plant compactness. Plants that were healed in chambers with lower RH (70%) were shorter and had less biomass than the ones that were healed in high humidity (90%) conditions. The low humidity chamber, however, showed similar transplant quality to plants in the optimal conditions described above. However, both the low humidity chamber (70%) and 10-day high humidity chamber (90%) produced less compact plants than plants from the optimal conditions (Vu et al., 2013).

Masterson et al. (2016a) reported that removing approximately 75% of the scion leaves during the grafting procedure increased graft survival rates. Leaf removal of the scion can help the plant tolerate water stress immediately post-grafting and can subsequently increase grafting success (Bumgarner and Kleinhenz, 2014; Masterson et al., 2016a). The act of removing scion leaves and grafting could invoke a plant response to wounding and/or water stress, and AR formation during the graft healing process could be impacted by this procedure as well (Guan et al., 2012).

The process of cutting the scion completely from its rootstock during splice grafting is similar to that of vegetative propagation, in which a plant part is excised and AR is promoted. Much of the literature about AR is related to vegetative propagation, in which AR is a desired process. Extensive research observing AR formation has reported that the plant hormone auxin, which is produced in the shoot meristem of young leaves and cotyledons, travels basipetally and accumulates at the wounding site of vegetatively-propagated plants (Katsumi et al., 1969; Maldiney et al., 1986; Nordström and Eliasson, 1991). Among the many functions of auxin is to promote xylem tissue regeneration and AR formation. Propagators wanting to promote AR apply auxin to the severed end of a plant cutting. (da Costa et al., 2013; Kevers et al., 1997). High auxin to cytokinin ratio in plant tissues is associated with AR formation (da Costa et al., 2013; Kevers et al., 1997; Katsumi et al., 1969; Maldiney et al., 1986; Nordström and Eliasson, 1991). Other plant hormones such as ethylene, abscisic acid, and jasmonic acid are known to interact with auxin to either promote or inhibit AR (da Costa et al., 2013). Although a complete understanding of all hormones and other phytochemicals associated with AR formation is still unclear, there is ample evidence that auxin is involved (da Costa et al., 2013).

Removing leaves may impact AR formation in grafted tomato because auxin is produced in young leaves (Katsumi et al., 1969; Maldiney et al., 1986; Nordström and Eliasson, 1991). Therefore, our hypothesis is that leaf removal could reduce the formation of AR in the scion of grafted tomato. However, the loss of leaf area and corresponding carbohydrate production may also negatively impact transplant quality, growth, and maturity. Bumgarner and Kleinhenz (2014) discuss using leaf removal for tomato grafting to decrease transpiration, but also note that it would increase wounding sites open for possible disease contamination as well as reduce photosynthesis and carbohydrate production during the post-grafting acclimation period.

Leaf removal could be a valuable technique for small-scale propagators who are wishing to graft their own tomato plants, but it is not clear how this practice affects the transplant quality and early plant growth of grafted plants. Therefore, the objectives of the studies in this report were to: (i) investigate how scion leaf removal affects the formation of adventitious roots, (ii) determine the impact of scion leaf removal on grafted transplant quality and (iii) identify the effect of scion leaf removal on early plant growth and development post-grafting.

2. Materials and methods

In order to address the three research objectives, two different, but complementary greenhouse studies were conducted during Spring 2014 at the Kansas State University Olathe Horticulture Research and Extension Center (OHREC). The goal of the first study

was to identify the effect of scion leaf removal on AR formation (objective 1) and the influence of leaf removal on transplant quality (objective 2). The aim of the second study was to measure plant growth (objective 3) and further develop information on the impact of leaf removal on transplant quality (objective 2). The first study was repeated three times whereas the second study was performed once due to the large amount of greenhouse space and labor required for plant growth assessment. Each individual experiment was replicated independently (n = 4) in both studies.

Both studies were conducted in a Quonset-style greenhouse with 10-mm twin-wall polycarbonate walls and a double-layer, 6-mil polyethylene film roof. Hybrid tomato 'BHN 589' (BHN Seed; Immokalee, FL) is often utilized for on-farm high tunnel trials as a grower-selected cultivar (Louws et al., 2010; Masterson et al., 2016b) and was used as the scion for this study. 'Maxifort' (De Ruiter; St. Louis, MO) was selected as the rootstock, and is a commercially-available rootstock and has been reported on in numerous studies with grafted tomato in the United States (Louws et al., 2010; Masterson et al., 2016b; Rivard and Louws, 2008; Rivard et al., 2010a,b).

During the course of all greenhouse experiments, tomato transplants were exposed to two different growing environments. For seed germination, transplant production, and post-grafting growth greenhouse environmental conditions ranged from 28 °C (day) to 18 °C (night) and plants were fertilized weekly with an application of Peter's Excel 15-5-15 Cal-Mag (Everris NA Inc.; Dublin, OH) water soluble fertilizer at a rate of 150 ppm N. When plants were moved into the healing chamber, the environmental conditions within the greenhouse changed. The healing chambers were located in a different greenhouse, and the temperatures ranged from 24 °C to 18 °C (day) and 28 °C to 21 °C (night). Plants were not fertilized while in the healing chambers. It is not typical for a propagator to place nongrafted plants in a healing chamber as the low light levels would promote elongated plants, which may detract from the quality of the transplant as defined by Vu et al. (2013). Therefore, while the grafted plants were in the healing chamber, the nongrafted plants were kept in a cool greenhouse with temperatures set at 13 °C–30 °C to slow plant growth while grafted plants were healed in the chamber for 10 days. After 10 days, grafted and nongrafted plants were placed back in standard growing conditions outlined above for the remaining time of the experiments.

Scion and rootstock were sown in Fafard Germination Mix media (Conrad Fafard Inc.; Agawam, MA) in 30 cm by 30 cm seedling trays on 19 March and 21 March, respectively, to accommodate for the variable germination speed of scion and rootstock cultivars. On 1 April, all seedlings were transplanted into 50-cell propagation trays using Fafard 3B Mix (Sun Gro Hort Canada Ltd.; Seba Beach, AB Canada). The plants were grown for and additional 12–14 days after transplanting and prior to being grafted to allow the rootstock and scion to grow to the appropriate stem diameter for splice grafting.

Adventitious Root Formation Study. Three levels of scion leaf removal were implemented, which included 0% (standard), 50%, and 90% (Fig. 1A). Cotyledons were left intact and attached as recommended (Bumgarner and Kleinhenz, 2014). Plants that underwent 50% and 90% leaf removal treatments had a portion of their leaves removed using florist's scissors. Leaves were removed immediately prior to grafting with care not to disturb the apical meristem or cotyledons. Leaf removal was performed by one person to reduce any bias across the treatments. The scion was then grafted onto 'Maxifort' rootstock using the splice grafting method (Rivard and Louws, 2011) with each individual grafter responsible for complete replications in order to prevent any grafting bias. The three repetitions of the AR formation study were replicated independently (within each chamber) and initiated (grafted) one day apart. Therefore, tomato plants were grafted on 13 April, 14 April, and 15 April and immediately placed into the shade, plastic, and humidifier chambers, respectively. Grafted plants were placed in a healing chamber arranged in a randomized complete block design with 4 blocks and 20 plants for each experimental unit (Fig. 1B). The experiment was conducted in three different chambers: in a shade chamber, a plastic-enclosed chamber, and a humidified chamber (Fig. 1C–E), which are described below.

The experiment was repeated in three different healing chambers in order to make general observations about different types of chambers that may be utilized by propagators. In each experimental run, the treatments were replicated independently within each chamber (n = 4). The three chamber designs were similar to Masterson et al. (2016a) and included the following: 1) shade cloth only (shade); 2) shade cloth and polyethylene film (plastic); and 3) shade cloth, polyethylene film, and a cool-mist humidifier (humidifier) (Fig. 1C–E). The chambers were constructed with 2.5 × 15 cm plastic lumber that was attached with screws to make a 120 × 90 × 15 cm base frame. Holes were drilled into the top edge of the plastic board frame that were approximately 60 cm apart and three 9-gauge wires were inserted into the holes and hooped over the frame creating a chamber that was approximately 22.5 cm in height at the center/peak. Both plastic and humidifier chambers were enclosed using 4-mil clear plastic sheeting and included three, round 10 cm-diameter openings that were located on top at each end to prevent excess heat build-up. The humidifier chamber had the same design as the plastic chamber and in addition a cool-mist humidifier (SU-2000, Sunpentown, City of Industry, CA) delivered vaporized water through a 5 cm polyvinylchloride (PVC) pipe to the enclosed chamber. Three removable layers of 50% shade cloth (SunBlocker Premium, Growers Supply; Dyersville, IA) were placed on top of each chamber and one layer of 50% shade cloth was fixed to the rafters of the greenhouse. Standard nursery (web) trays were turned upside down and placed on the bottom of the plastic and humidifier chamber to hold grafted plant trays out of standing water (1–2 cm) on the floor of the chambers. Fresh water was added in the bottom of plastic and humidifier daily to maintain at least 1 cm of water in the bottom of the chamber.

Temperature and RH within the healing chambers were recorded using a data logger (EL-USB-2-LCD, Lascar Electronics, Erie, PA) that was placed in the middle of each of the three chambers. Although these data were not replicated, it provided descriptive information about the microclimate within each chamber. During the first 7 days post-grafting, the average temperature and RH in the shade, plastic, and humidifier chambers were 19.8 °C and 68%; 21.6 °C and 95%; and 22.1 °C and 95%, respectively. After 3 days of healing at low light, plants were slowly acclimated to higher light by removing layers of shade cloth; however, shade cloth was left on during sunny days. By day 7, all shade cloth was removed from chambers and polyethylene film sides were lifted from plastic and humidifier chambers to allow the relative humidity to decrease. On day 8, the polyethylene film was completely removed from the plastic and humidifier chambers. For the humidifier chamber, the cool-mist humidifier was set to high to increase humidity quickly in the chamber during the first day, but was turned down to low on day 2 and was turned off on day 7. For all treatments, plants were moved to full light and standard greenhouse environmental conditions on day 10.

On days 10, 17, and 24 days post-grafting, all plants were rated for AR formation on the scion using a rating system developed based on preliminary experiments with grafted transplants. The ratings start at zero, indicating no AR formation or root initials observed on the epidermis. Based on the number of trace roots (<2 mm), small roots (≥2 mm), medium root (≥5 mm) and/or large roots (≥10 mm), each plant was rated on a scale of 0 to 10 where: 0 = none; 1 = trace root(s) only; 2 = ≥ 1 small root(s) and any trace root(s); 3 = 1 medium root and any small or trace root(s); 4 = multiple medium

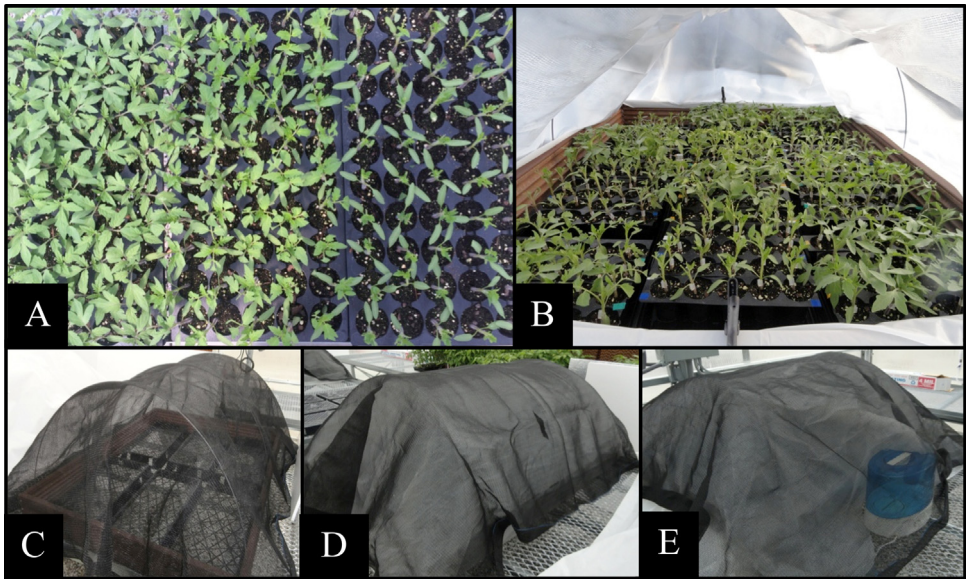

Fig. 1. (A) Leaf removal of the scion, 0%, 50%, and 90% true leaf removal (shown left to right), was applied to scion var. BHN 589 just prior to grafting to rootstock 'Maxifort'. (B) Once the plants were grafted, they were placed inside each chamber as a RCBD experiment with 20 plants per unit and four replications. Experimental repetitions were conducted in healing chambers with (C) shade cloth alone (shade), (D) shade cloth and polyethylene film (plastic), and (E) the plastic chamber with a cool-mist humidifier (humidifier).

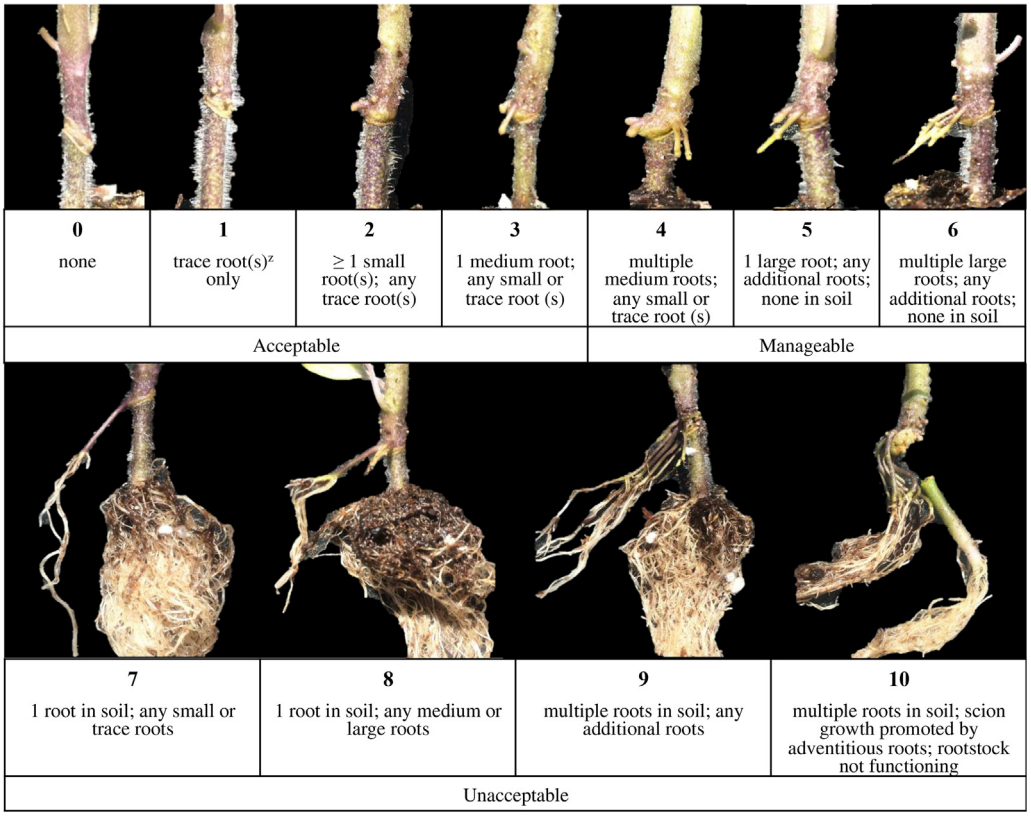

Fig. 2. A rating scale 0–10 was used to describe the degree of adventitious root formation from the scion and determine the marketable quality of the transplant. Pictures were taken on day 24 post-grafting (trace root <2 mm ≤small root <5 mm ≤ medium root ≤ 10 mm ≤ large root).

roots and any small or trace root(s); 5 = 1 large root and any additional roots but none in the soil; 6 = multiple large roots and any additional roots but none in soil; 7 = 1 root in soil and any small or trace root(s); 8 = 1 root in soil and any medium or large root(s); 9 = multiple roots in soil and any additional roots; and 10 = multiple roots in soil causing promotion of scion growth by AR and failure of rootstock growth) (Fig. 2). Plants with an AR rating of 1 to 6 had no AR in contact with the soil/media. During data analysis, the numerical ratings were grouped into three categories (acceptable, manageable, and unacceptable), based on the overall effect of AR formation on transplant quality and marketability. Plants with ratings 0–3 had insignificant adventitious rooting and were consid-

ered acceptable for use. Plants with ratings 4–6 were categorized as manageable because a grower producing a small number of their own transplants could trim the roots before planting to ensure no AR made soil contact after planting. Plants with ratings of 7–10 were considered unacceptable. Plants with an unacceptable rating represent an unmarketable plant for a propagator as well as an unusable plant for a grower producing their own transplants. Twenty-four days after grafting, all AR were removed from each scion and the fresh weight of AR was recorded for each treatment.

2.1. Post-grafting plant growth study

Plants for the second study were seeded, grafted, and given post-grafting care identical to the shade chamber experiment mentioned in the first study. Grafted plants that were used for the plant growth study had little to no AR. Although the objective of the study was to compare the impact of leaf removal on grafted plant growth, nongrafted 'BHN 589′ tomato plants were incorporated into the experimental design as an additional comparison. Nongrafted plants were seeded on the same day as the grafted ones (19 March), and were placed in a cool greenhouse (13 °C–30 °C) for 10 days while the grafted plants were in the healing chamber in order to minimize growth. Otherwise, they were grown in the same manner. The low light levels and warm growing temperatures in the healing chambers encourage nongrafted plants to become elongated, which is not preferred by growers nor indicative of a typical nongrafted tomato transplant. It takes approximately 21 days post-grafting for a grafted tomato seedling to completely heal and be ready for transplant or sale (Bumgarner and Kleinhenz, 2014; Rivard et al., 2010b). Therefore, the measurements taken 24 and 31 days post-grafting are the most indicative of the ideal plant size for a propagator that wishes to sell or plant grafted transplants, while day 38, 45, and 52 post-grafting represented tomato early growth and development.

Each of the experimental units was comprised of 15 plants and was arranged in a randomized complete block design with 4 replications. Grafted and nongrafted plants (4 cm soil plugs) were transplanted into 10 cm pots on 8 May (26 days post-grafting) and then transplanted into 10 L containers on day 35. Three random plants within each experimental unit were destructively sampled on days 24, 31, 38, 45, and 52 post-grafting in order to measure plant growth. At each sampling date, three random plants per experimental unit were measured for leaf area, shoot biomass, root biomass, plant height, stem caliper, and flower count. Plants were severed at the soil level and plant height was measured to the apical meristem. Stem diameter measurements were taken 1 cm above the scion cotyledons using a 147 Digital Fractional Caliper (General Tools and Instruments LLC; New York, NY). Leaf area was measured with a LI-3100 Area Meter (Li-cor Inc., Lincoln, NE). All leaf and stem, and flower tissue were combined, dried at 70 °C for at least 72 h, and weighed to assess shoot biomass. Plant roots were carefully washed to remove debris, dried and weighed. Flowers were categorized into three stages: buds (still closed), yellow (showing any sign of yellow), and pollinated (swelled ovary) and recorded.

2.2. Statistical analysis

All data were analyzed using PROC GLIMMIX (SAS 9.2; SAS Institute, Cary, NC USA). Least significant differences for post-grafting AR ratings on days 7, 17, and 24, as well as the fresh weight of AR excised from scion on day 24, were analyzed independently and compared with Tukey's method where $\alpha = 0.05$. Although destructive sampling was utilized, the transplant and early growth experiments were dependent on time within a shared (greenhouse) environment. Therefore, PROC GLIMMIX (SAS 9.2; SAS Institute, Cary, NC USA) was used to analyze stem diameter, height, leaf area, root and shoot biomass, shoot-to-root ratio, compactness, and flower counts separately on days 24, 31, 39, 45, and 52 post-grafting. Growth parameters at days 24 and 31 after grafting were considered to be the transplant quality assessments. Growth parameters at days 38, 45, and 52 days after grafting were considered to be the "early growth" assessments. Plant compactness was calculated by dividing the shoot biomass by plant height. Based on the residuals of each data set, the best model for covariance structure was selected for each parameter. For stem caliper and plant height, we used first-order autoregressive. For leaf area, root biomass, flower count, and compactness, we used heterogeneous compound symmetry. For shoot biomass and shoot-to-root ratio, we used heterogeneous first-order autoregressive. Furthermore, flower count data (y), which included several 0 count data, was transformed where $y^* = \sqrt{y + \frac{3}{8}}$ for analysis using the Poisson model. Furthermore, we used the Bonferroni method to compare treatment LSDs for each independent growth parameter separately on each sampling day with $\alpha = 0.05$.

3. Results

3.1. Effect of leaf removal on adventitious root formation

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

3.2. Effect of leaf removal on transplant quality

Transplant quality for days 24 and 31 post-grafting was determined by the AR formation ratings in the first experiment and growth parameters (stem diameter, height, shoot and root biomass, and compactness) in the second experiment as defined by Vu et al. (2013). When compared to the standard (0% leaf removal) splice grafting method, removing 90% of the scion leaf tissue decreased the proportion of plants that had unacceptable levels of AR in all three chamber environments (Fig. 3). A similar comparison with the 50% leaf removal method indicated the same trend in the shade chamber (Fig. 3). Post-grafting growth measurements indicated that plants with 90% leaf removal method decreased transplant quality in regards to leaf area, shoot biomass, root biomass, and compactness when compared to the standard method. However, the 50% leaf removal method had similar transplant quality when compared to 0% leaf removal except for root biomass on day 24 and leaf area on day 31 (Table 2; Fig. 4).

When healed in the shade chamber, 97% and 99% of the grafted plants with the 50% and 90% leaf removal treatments, respectively, were considered acceptable 24 days after grafting. Similarly, 1–3% were considered manageable, and 0% were considered unacceptable (Fig. 3A and B). In contrast, the standard method (0% leaf removal) had 71% acceptable, 19% manageable, and 10% unacceptable grafted transplants (Fig. 3A). In the plastic and humidifier chambers, 90% leaf removal also had high acceptable rates of 98% and 95%, respectively, and 0% unacceptable grafts (Fig. 3B and C). However, plants with 50% leaf removal in the plastic and humidifier chambers had acceptable, manageable, unacceptable rates of 65%, 26%, 9% and 60%, 31%, 9%, respectively (Fig. 3B and C). The standard method of 0% leaf removal resulted in the lowest proportion

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

	Average AR Rating (per plant)[2,5]						Fresh Weight of AR (mg/plant)[4]	
Treatment[1]	Day 10[4]		Day 17		Day 24			
Shade Chamber[3]								
0% LR	1.62	a[6]	2.21	a	2.49	a	38.13	a
50% LR	0.31	b	0.45	b	0.55	b	0.76	b
90% LR	0.05	b	0.12	b	0.16	b	0	b
Plastic Chamber								
0% LR	2.99	a	3.47	a	3.52	a	49.07	a
50% LR	2.18	a	2.51	a	2.6	a	17.33	a
90% LR	0.59	b	0.75	b	0.81	b	1.63	b
Humidifier Chamber								
0% LR	4.1	a	4.11	a	4.19	a	39.79	a
50% LR	2.73	ab	2.94	a	3.03	a	22.41	a
90% LR	0.91	b	1.04	b	1.1	b	1	b

[1] Treatments include percentage of leaf removal (LR) removed from the scion ('BHN 589') during grafting onto rootstock 'Maxifort'.
[2] Experimental design including RCBD with 20 plants in each experimental unit.
[3] Experiment was performed in 3 different chambers described in Fig. 1.
[4] Data was collected 10, 17 and 24 days post-grafting. On day 24, adventitious roots (AR) were excised and weighed.
[5] Adventitious root (AR) ratings were determined using a scale from 0 to 10 (shown in Fig. 2) based on size and length of roots 10, 17, and 24 days post-grafting.
[6] Different letters represent significance based on Tukey's $\alpha = 0.05$. Data was analyzed independently for chamber and day.

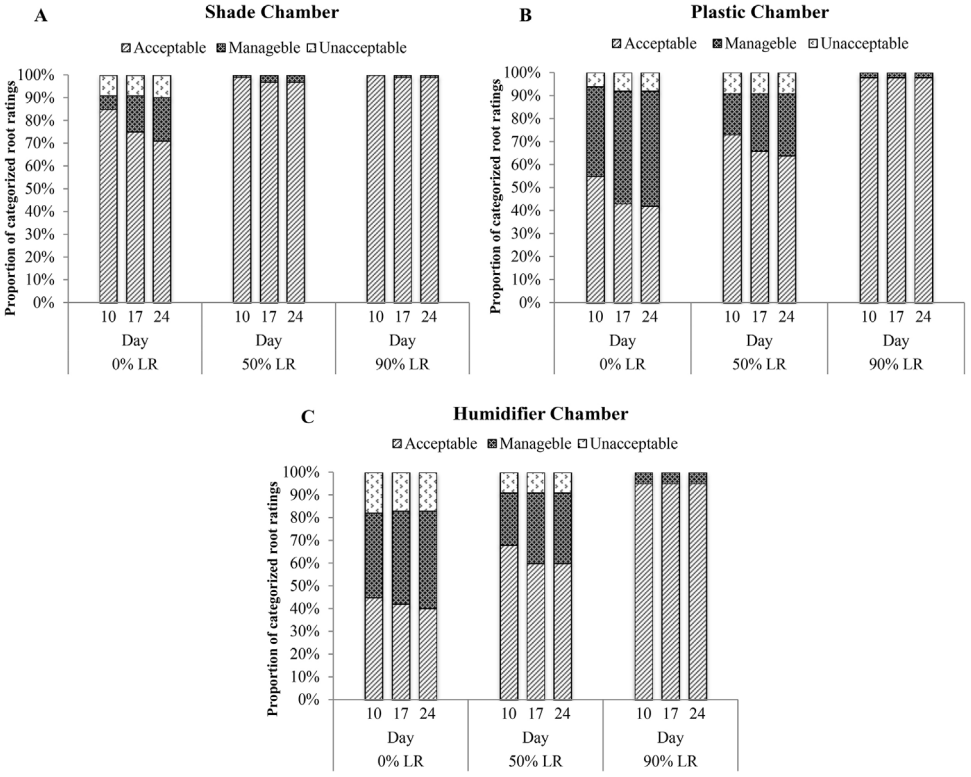

Fig. 3. Proportions of the quality of grafted tomato transplants determined by AR formation on days 10, 17, and 24 days post-grafting in (A) shade, (B) plastic, and (C) humidifier chamber. Using the scale in Fig. 2, ratings of 0–3 were categorized as acceptable quality; ratings of 4–6 were manageable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

of acceptable ratings in the plastic and humidifier chambers as well as the highest percentage of manageable and unacceptable rates of AR (Fig. 1B and C).

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

Using the standard technique of 0% leaf removal, grafted transplants produced significantly less shoot biomass than nongrafted 'BHN 589′ transplants on both day 24 and 31 as well as smaller stem caliper and compactness on day 31 ($\alpha = 0.05$; Table 2; Fig. 4A, C and F). Furthermore, when comparing to nongrafted transplants, grafted plants with 50% leaf removal decreased stem diameter,

Table 2
Plant growth measurements of nongrafted tomato and grafted tomato grown in a greenhouse at the Olathe Horticulture Research and Extension Center with varying levels of leaf removal utilized during grafting.

Treatment	Stem (mm)[5]		Height (cm)		Leaf Area (cm^2)		Shoot Biomass (g)		Root Biomass (g)		Shoot:Root Ratio		Compactness (mg/cm)	
Day 24[4]														
0% LR[1,2,3]	4.5	ab[6]	15.4	ab	110	a	0.84	b	0.17	ab	5.02	ab	55.1	ab
50% LR	4.2	b	13.8	ab	91	ab	0.67	bc	0.11	c	6.98	a	48.4	bc
90% LR	4.0	b	11.7	b	77	b	0.48	c	0.13	bc	3.65	b	41.2	c
Nongrafted	5.1	a	16.9	a	117	a	1.11	a	0.19	a	5.80	ab	65.2	a
Day 31														
0% LR	4.9	b	18.2	ab	188	a	1.20	b	0.46	ab	2.62		65.9	b
50% LR	4.9	b	16.4	ab	170	b	1.07	b	0.40	b	2.75		65.3	b
90% LR	4.4	b	15.1	b	149	c	0.86	c	0.31	c	2.76		57.0	c
Nongrafted	5.7	a	19.0	a	174	ab	1.45	a	0.50	a	2.89		76.2	a
Day 38														
0% LR	6.4	ab	22.5		343	a	2.76	a	0.87	a	3.21		123.4	
50% LR	6.1	bc	21.0		313	a	2.55	ab	0.76	ab	3.35		121.6	
90% LR	5.6	c	19.5		260	b	2.11	b	0.61	b	3.45		108.5	
Nongrafted	7.1	a	23.1		267	b	2.66	a	0.87	a	3.13		115.2	
Day 45														
0% LR	8.8	a	41.9	a	1850	a	10.35		2.88	a	3.61	b	246.8	a
50% LR	8.2	ab	39.0	ab	1873	a	9.97		2.74	a	3.64	b	256.3	a
90% LR	8.1	b	36.5	b	1676	a	9.18		2.02	b	4.57	a	251.7	a
Nongrafted	8.9	a	39.4	ab	1335	b	8.21		1.95	b	4.22	ab	208.6	b
Day 52														
0% LR	10.3	a	58.8	a	3387	a	37.30	a	7.39		5.10		633.6	
50% LR	9.9	ab	57.0	ab	3118	ab	35.15	ab	6.79		5.26		616.9	
90% LR	9.5	b	54.3	b	3216	a	33.65	ab	6.39		5.41		619.9	
Nongrafted	10.4	a	58.6	a	2696	b	30.45	b	5.78		5.27		519.7	

[1] Treatments include 'BHN 589' tomato plants with 0%, 50%, and 90% leaf removal (LR) grafted onto 'Maxifort' rootstock and 'BHN 589' nongrafted plants.
[2] Scion and nongrafted plants were planted on 19 March, two days before rootstock plants. All plants were grown under typical greenhouse optimal growing conditions for tomato (18 °C–28 °C) except during days 1–10 post-grafting.
[3] For days 1–10 post-grafting (using splice-grafting method), grafted plants were placed in shade cloth only healing chamber (avg. 68% RH and 20 °C) while nongrafted plants were placed in a non-shaded but cooler environment (13 °C–30 °F).
[4] Experiment was arranged in a RCBD with 4 blocks and 15 plants in each experimental unit. Destructive sampling of 3 plants per unit occurred 24, 31, 38, 45, and 51 days post-grafting.
[5] Data was analyzed using a mix model with selected covariance structure based on residuals for each growth parameter: stem and height data used first-order autoregressive; leaf area, root biomass, and compactness data used heterogeneous compound symmetry and shoot biomass and shoot-to-root ratio used heterogeneous first-order autoregressive.
[6] Different letters show significant differences between values when using the Bonferroni method to compare LSDs independently of day with $\alpha = 0.05$.

shoot and root biomass, and compactness. Grafted transplants with that underwent the 90% leaf removal method had reduced transplant quality growth measurements ($\alpha = 0.05$; Table 2; Fig. 4).

3.3. *Effect of leaf removal on early plant growth and development*

The effect of leaf removal on early plant growth and development for grafted transplants was determined by comparing growth measurements and flower counts on day 38, 45, and 52 for plants with 50% or 90% leaf removal to plants with 0% leaf removal (standard). Furthermore, plant growth data was plotted in line graphs (Fig. 4) to show the profile of the data over time, but is also provided in a table for presentation of the statistical analysis (Table 2). Although the compactness of all grafted plants were similar, 90% leaf removal of the scion reduced stem caliper, height, leaf area, and shoot and root biomass (Table 2; Fig. 4). In contrast, plants grafted with 50% leaf removal had similar early plant growth compared to the standard grafting method (0% leaf removal). During sampling during the early growth period, grafted plants surpassed nongrafted plants in leaf area (day 38, 45, and 52), shoot (day 52) and root biomass (day 45), and compactness (day 45) (Table 2; Fig. 4C–F).

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

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

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

In addition to leaf area, shoot and root biomass were also reduced on day 38 for plants with 90% leaf removal grafting method compared to both 0% leaf removal grafting method as well as the nongrafted plants ($\alpha = 0.05$; Table 2; Fig. 4D and E). On day 45, shoot biomass was similar among all treatments. However, root biomass for grafted plants with 90% leaf removal was significantly lower

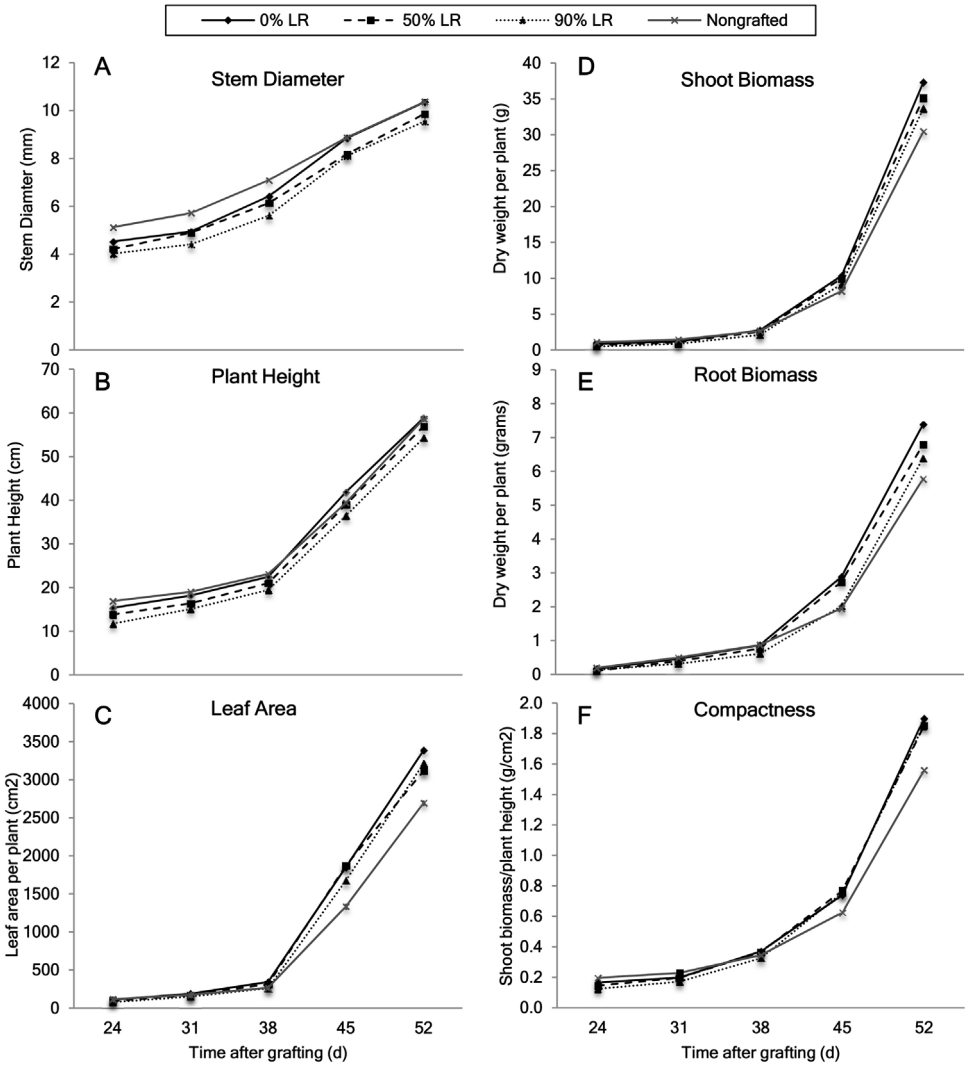

Fig. 4. Nongrafted 'BHN 589' tomato plants and grafted plants with 'BHN 589' scion and 'Maxifort' rootstock were treated with 0%, 50% or 90% leaf removal of scion leaf tissue at the time of grafting and placed in a shade cloth only healing chamber (avg. 68% RH and 20 °C) for 10 days and then arranged as a RCBD experiment that was conducted in a greenhouse at the Olathe Horticulture Research and Extension Center. On days 24, 31, 38, 45 and 52 post-grafting, 3 randomly sampled plants for each treatment for each of the 4 blocks were destructively measured for the following growth parameters: (A) stem diameter; (B) plant height; (C) leaf area; (D) shoot biomass; (E) root biomass; and (F) compactness.

than the grafted plants with 50% and 0% leaf removal ($\alpha = 0.05$; Table 2; Fig. 4D and E). Nongrafted plants also showed reduced root biomass compared to plants with 0% and 50% leaf removal on day 45, but on day 52 all grafted plants (0%, 50%, and 90% leaf removal) and nongrafted plants had similar root biomass (Table 2; Fig. 4E). Overall, the treatments consistently showed comparable shoot-to-root ratios throughout the sampling period. One exception to this trend was on day 45, when grafted plants with 90% leaf removal showed a higher ratio than the ones that were grafted with the 0% (standard) and 50% techniques, which was mostly likely due to its low root biomass (Table 2).

Measurements on sampling days 38, 45 and 52 indicated that all grafted plants (0%, 50%, and 90% leaf removal) had similar plant compactness and were the same (day 38 and 52) or more compact (day 45) than nongrafted plants ($\alpha = 0.05$; Table 2; Fig. 4F). No flowers were observed on any plants until 38 days post-grafting (Fig. 5). Pollinated flowers were counted on day 45 for plants grafted with the 0% leaf removal method as well as the nongrafted plants. On day 52, all treatments had pollinated flowers. Because the counts were low for the various maturity levels (buds, yellow, and pollinated), the data were combined for statistical analysis of total flower count between treatments on days 38, 45, and 52 (Fig. 5). All treatments were found to be similar for days 38 and 45 (Fig. 5). On day 52, the plants that underwent 50% leaf removal had the highest mean number of flowers and was significantly higher than the nongrafted plants, but was similar to the other grafted treatments ($\alpha = 0.05$; Fig. 5).

4. Discussion

Our studies indicate that scion leaf removal can affect AR formation, transplant quality and early growth of grafted tomato transplants. Removing a large portion of the scion leaf area (90%) during the grafting procedure consistently decreased AR formation from the scion, which resulted in high rates of acceptable transplants in relation to AR. Moreover, removing a smaller proportion of scion leaves (50%) successfully reduced AR formation and produced a high rate of acceptable transplant in regards to AR as well. Categorized AR formation rating data showed a trend where the grafted plants with significantly lower ratings had a higher proportion of acceptable grafts, which indicated superior transplant quality as outlined by Vu et al. (2013). In all three healing chamber

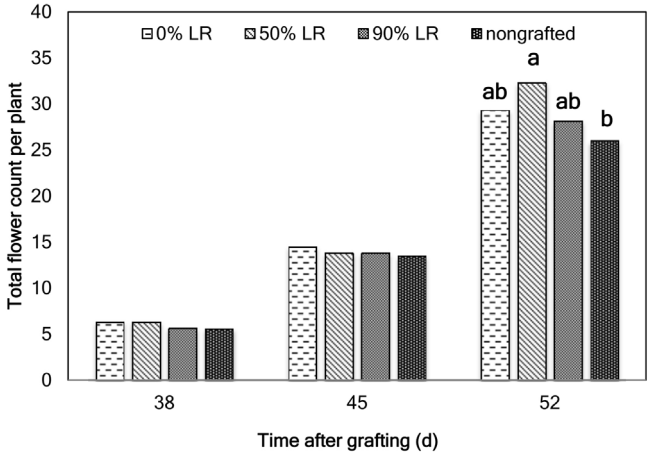

Fig. 5. Total flower count of plants grafted with 0%, 50%, and 90% leaf removal (LR) as well as nongrafted plants when seeds are planted on the same day. Flower count was determined using 3 randomly sampled plants for each treatment for each of the 4 blocks in a RCBD experiment that was conducted in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

environments, the standard method of grafting (0% leaf removal) had the highest AR ratings as well as large proportions of unacceptable (8%–17%) and manageable (19%–50%) grafted transplants showing potential to lose successful grafted transplants as a result of excessive AR formation.

The shade chamber had the lowest RH, suggesting that humidity had an influence on the increase of AR formation on plants with 50% leaf removal in the various microclimates. Extension publications that discuss healing chamber management have attributed overly humid chambers or extended time in a humid chamber to cause AR formation on grafted scion (Johnson et al., 2011). Our results support this claim, although our data is not replicated properly to verify it statistically. However, plants that were grafted with the 90% leaf removal method had significantly lower AR formation than 0% leaf removal in both low and high humidity chamber environments, suggesting that in addition to RH, leaf removal may influence the plant's initiation of AR following grafting. Therefore, it could be theorized that AR initiation on tomato scion is linked with the plant's endogenous reaction to stress and/or wounding and further AR growth following initiation may be exacerbated by environmental factors like humidity.

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

Wounding has been reported to promote AR as the plant hormone auxin accumulates at the basal end of severed stems during vegetative propagation (Katsumi et al., 1969; Maldiney et al., 1986). Auxin flows basipetally and when the root system is completely removed, auxin that is produced in the young leaves and meristematic tissue above the wound accumulates at the basal end (da Costa et al., 2013). Contrary to auxins, cytokinins are mostly produced in the root meristem and transported apically (Maldiney et al., 1986). A ratio of high levels of auxin and low levels of cytokinin is most notably associated with the formation of AR (da Costa et al., 2013). Results from Maldiney et al. (1986) reported that in the first three days after excising the root system from a tomato cutting, auxin levels at the base of the tomato cutting rose while cytokinin levels dramatically dropped after 24 h. and stayed low. It is interesting to note that the rootstock plants in our study, which underwent the same wounding process and healing chamber environment as the scion, did not form AR on hypocotyls in any of the experiments. Because of its excised shoot, the rootstock would most likely have more cytokinins accumulate at the graft wound than auxin resulting in low AR formation. In addition, the leaves, cotyledons, and apical meristem above the wounding site could be a source of auxin that is transported toward the graft union (Katsumi et al., 1969). Katsumi et al. (1969) showed that the removal of different proportions of cotyledon surface area while leaving the apical meristem intact on cucumber cuttings reduced auxin levels at basal site and decreased AR formation. Similarly, removing leaves on young tomato seedlings prior to grafting may help reduce the amount of auxin transported to the wound site. If removing leaves decreases the amount of auxin accumulated at the graft site on the scion, it could result in less AR induction and initiation as seen in our studies.

Although our experimental design was not replicated in the proper manner to compare chamber environment effects and/or interactions with leaf removal on AR development, we can note observed trends between the particular environments and compare them to previous studies. Both the greenhouse chamber designs utilized were identical to those utilized by Masterson et al. (2016a) who noted that the shade cloth chamber had significantly lower humidity and temperature than both plastic and humidifier chambers. The RH of the shade, plastic, and humidifier chambers utilized in this study were: 65%, 95%, and 95%, respectively, and similar chambers had mean values of 69%, 85%, and 91% RH, respectively (Masterson et al., 2016a). In addition, healing chamber studies by Johnson and Miles (2011) reported that chambers with shade cloth only had 53% RH and polyethylene film-covered chambers with and without humidifiers had 82% and 98% RH, respectively. Although our experiment was not replicated in a manner to test this question, the microclimate found within each healing chamber was similar to others that have been reported.

Further results from plant growth study revealed that transplant quality and early plant growth can be affected by leaf removal depending on the proportion of leaf area removed and length of time post-grafting. Although, the 90% leaf removal method resulted in similar transplant height and stem diameter to other grafted plants (0% and 50% leaf removal), its reduction in leaf area and shoot biomass created less compact transplants. However, the continued growth of the grafted plants with the 90% leaf removal method resulted in significantly shorter plants with smaller stems but similar biomass and compactness as the ones grafted using the standard method. On the other hand, when only removing 50% of scion leaf area, grafted plants resulted in similar transplant quality as well as early growth to the standard method of 0% leaf removal except for reduced root biomass and leaf area on day 24 and 31, respectively.

One of the potential disadvantages of the leaf removal method is that removing scion leaves decreases photosynthetic leaf area, which would limit carbohydrate production during healing (Bumgarner and Kleinhenz, 2014). It is likely that reducing 90% of

the leaf area during grafting in our studies also reduced the amount of subsequent plant growth, due to lower carbohydrate availability in the plant as compared to the plants with 0% and 50% leaf removal. In addition, removing 90% of the leaf tissue may affect plant growth traits, such as stem thickness, compactness, and optimum shoot to root biomass than farmers desire in high quality transplants for purchase by local propagators. Depending on the desired transplant quality attributes, propagators using the leaf removal technique to increase grafting success and reduce AR formation may consider extending transplant production time in order to strive for a higher quality transplant. Furthermore, our data suggests that it may benefit propagators using shade only chambers to keep at least 50% leaf area in order to produce higher quality grafted transplants and promote early growth.

Flower counts were found to be similar for all leaf removal treatments compared to the standard method of grafting. Interestingly, plants grafted with the 50% leaf removal method had a higher flower count than the nongrafted plants on day 52. However, more research is needed to further clarify this phenomenon. These data suggest that leaf removal and grafting may have little influence on the timing of reproductive development in tomato. However, this greenhouse study occurred in a controlled environment and only included data from the first three weeks of reproductive development without fruit harvest data. Furthermore, our studies only used one scion and rootstock combination. The same pairing of 'BHN 589' scion and 'Maxifort' rootstock has shown to increase total fruit number and scion biomass at the end of a growing season in high tunnel trials with little to no disease pressure (Masterson et al., 2016b). Masterson et al. (2016b) also showed that removal of shoot apical meristem (aka shoot removal) of grafted tomato transplants reduced early yield when planted in high tunnels. Season extension and early production is a major focus for many growers, particularly those producing tomato fruit in high tunnels (Carey et al., 2009; Everhart et al., 2009; Galinato and Miles, 2013; Sydorovych et al., 2013; Waterer, 2003). Therefore, a reduction in early production could decrease profitability because of the potential loss of premium markets (Rivard and Louws, 2008; Masterson et al., 2016b).

Although we used nongrafted plants to compare transplant quality and early growth of grafted plants with varying levels of scion leaf removal, they were more for observation and considered less valuable in evaluating the leaf removal method than comparisons with the standard grafting method (0% leaf removal). It should be noted that in this study, nongrafted plants were placed in a cool greenhouse (55 °F–85 °F) for 10 days while the grafted plants were in the healing chamber. This experimental protocol minimizes growth by the nongrafted plants while the grafted plants heal and allows for seeds of both treatments to be sown and subsequently transplanted on the same day. However, this process affects plant growth of the nongrafted plants in that cool nighttime temperatures keep the plants short with thick stems and compact growth as seen in our results on days evaluating for transplant quality and may not be typical for nongrafted transplant production.

5. Conclusions

Because grafted transplants represent a much higher investment to propagators than nongrafted plants (Barrett et al., 2012; Lewis et al., 2014; Rivard et al., 2010b), grafting success rates and growing high quality grafted transplants is extremely important to maximize profitability. Leaf removal during grafting can increase grafting survival rates (Masterson et al., 2016a) and in this study, it reduced AR development in three different healing chamber environments. Any simple technique, such as leaf removal, that increases survival rates and transplant quality is advantageous for small-scale growers that wish to propagate their own grafted tomato plants. Furthermore, this method may better facilitate the use of healing chambers with low RH as utilized in previous studies (Johnson and Miles, 2011; Masterson et al., 2016a).

A clear question relates to the economics of leaf removal and how it would affect overall grafted transplant production costs with added labor for trimming. Current production cost budgets that have been reported for grafted tomato transplants estimate grafting success at 90% (Rivard et al., 2010b), which is lower than the grafting success rates found in these studies. Grafted plants with an unacceptable level of AR would be considered a loss and any level of AR reduces transplant quality (Vu et al., 2013) and therefore marketability. These losses reduce profit for propagators and represent opportunity costs for tomato growers that are grafting their own plants. Propagators may also find that tomato scions with trimmed leaves are easier to manage and less likely to dislodge from the grafting clip due to lower shoot weight and surface area. Research that investigates the economics of leaf removal and identifies both the costs and potential economic benefits of this technique would be valuable.

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

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