ABSTRACT

RIVARD, CARY LEE. Grafting for Open-field and High Tunnel Tomato Production. (Under the direction of Frank J. Louws and H. David Shew).

Due to the recent phaseout of methyl bromide and high demand for local and organic produce, tomato growers in NC require environmentally-sustainable soilborne disease management strategies for organic and conventional systems. Tomato grafting is popular worldwide, but it’s practical and economic relevance for US production is unknown. A research and extension program was initiated to evaluate the utility of tomato grafting for US growers. Field research trials were carried out in from 2007-2009 that included on-farm collaborations in organic and conventional systems. Several tomato rootstocks were identified that conferred resistance against three economically-important soilborne plant pathogens, including *Ralstonia solanacearum*, *Sclerotium rolfsii*, and *Meloidogyne* spp.

‘Big Power’ was highly effective at reducing root galling by *Meloidogyne* spp. beyond nongrafted, fumigated, and other hybrid rootstocks containing the *Mi* resistance gene. Other inter-specific rootstocks also provided host resistance against pathogens where commercial tomato hybrids are highly susceptible. In an on-farm trial in Alamance County, ‘Beaufort’ and ‘Maxifort’ showed high resistance to *Sclerotium rolfsii* whereas control treatments had 45-50% disease incidence, and similar results were seen in Sampson County with ‘Big Power’. ‘RST-04-105’ and ‘Dai Honmei’ showed partial resistance to *R. solanacearum* and this pathogen causes a particularly severe disease on tomato known as bacterial wilt. In two trials during 2009, *R. solanacearum* had killed >60% of the nongrafted plants at first fruit harvest and plants grafted with ‘Dai Honmei’ had 0% and 13% bacterial wilt incidence at the end of the season.
An important question concerning the relevance of grafting as a disease management tool is the potential benefit of vigorous rootstocks to increase yield in non-infested soils. If grafting is economically viable under environmental conditions that are not suitable for disease, then resistant rootstocks could be deployed preventatively to reduce risk of disease epidemics. Research was carried out at the Center for Environmental Farming Systems (CEFS) to test this hypothesis. Grafting with ‘Beaufort’ and ‘Maxifort’ rootstocks increased fruit yield in by 23% to 54% in open-field and high tunnel growing systems ($P<0.05$). In addition, the grafted plants provided yield benefits in the early part of the summer and the main effect of grafting was significant regardless of season length ($P<0.05$). Other nested studies in the CEFS trial included evaluation of ‘RST-04-105-T’ and ‘Big Power’ rootstocks and the manipulation of planting density. Interestingly, grafted plants grown at reduced planting densities had similar or higher fruit yield than nongrafted plants and this finding could help to alleviate economic constraints associated with the cost of grafted transplants.

In order to determine the physiological state of induced resistance associated with grafting, a quantitative PCR protocol was developed to monitor defense gene expression in tomato. Grafting elevated the expression of proteinase inhibitor II (PIN II), a defense gene related to the jasmonic acid pathway, and quantitative expression of PIN II was altered by rootstock genotype. Grafting is a highly effective management strategy that is now being readily adopted by NC tomato growers, and further clarification of induced resistance will enhance our application of host genetics in the field.
Grafting for Open-field and High Tunnel Tomato Production

by

Cary Lee Rivard

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Plant Pathology

Raleigh, North Carolina

2010

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DEDICATION

This work is dedicated to all of the family and friends that have provided me with so much support over the years. To my parents: whose continued love and support are indescribable. To my brother: who actually suggested that I completely disassemble a 1998 Toyota engine and then put it back together again. And of course, to my loving wife, Tia, and son, Samuel: We made it!
BIOGRAPHY

Cary Rivard was born in Kansas City, MO on June 26, 1981. He earned a B.S. degree from Truman State University (Kirksville, MO) in 2004, and Cary double-majored in Biology and Agricultural Science, with area specializations in plant physiology and horticulture, respectively. While at Truman, he found an interest in teaching and learning scientific and biologically-based farming principles. He also diversified his education through off-campus experiences including: Bailey Nurseries (Yamhill, OR), the Center for Environmental Farming Systems (Goldsboro, NC), and the Land Institute (Salinas, KS). Cary also spent his last 2 undergraduate years, as head brewer of a newly-established local microbrewery in Kirksville, where he learned the best answer to the common freshman biology class question: “when are we ever going to use this knowledge again?”

In 2004, Cary moved to Raleigh, NC and began to answer this question as a teaching assistant for the Biological Sciences Department. He obtained a graduate research assistantship in the Department of Plant Pathology in the summer of 2005, and received his M.S. degree in Plant Pathology in the spring of 2007 under the direction of Frank J. Louws. As a graduate student, Cary was active in the Plant Pathology Graduate Student Association, American Phytopathological Society, and also volunteered at several outreach events at the Center for Environmental Farming Systems. Cary also completed the Preparing the Professoriate professional development program in 2009, and presented at a number of grower workshops and other extension venues.
ACKNOWLEDGEMENTS

I would like to acknowledge everyone in my life that has helped me along the way, but the binding costs would be overwhelming. I would especially like to thank Dr. Frank Louws and everyone in the Integrated Disease Management Lab. Statewide field research takes an enormous effort, and it couldn't have been accomplished without the excellent help of Jim Driver, Rob Welker, Mike Carnes, Amy Keeter, and many others.

Numerous people are acknowledged within the chapters of this thesis, but I think it's also important to draw special attention to those that are listed as co-authors within each chapter. I would especially like to thank Dr. Mary Peet, Suzanne O'Connell, Chris Harlow, Olha Sydorovych, Raul Salinas, and Heike Winter Sederoff because their cooperation on these projects was instrumental to their success.

I would also like to thank my gracious on-farm collaborators including: Alex and Betsy Hitt, Ken Dawson, Stefan Hartmann, Chris Powell, Steve Groff, Kent Cochran, the Mtn. Hort. Crops Res. and Ext. Ctr. (Mills River, NC), Hort. Crops Res. Station (Clinton, NC), CEFS (Goldboro, NC) and everyone at the NCSU Phytotron (Raleigh, NC).

I especially want to thank my committee members and my co-advisor, Dr. H. David Shew, who allowed me to invade the sanctity of his classroom as a teaching assistant in PP 315 and who served as my teaching mentor for the PTP program. Last but not least, I sincerely appreciate the kind words, teaching, and support that I received from the students, faculty, and staff in the Department of Plant Pathology at NCSU.
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CHAPTER ONE

The following report

_Grafting tomato with inter-specific rootstock to manage diseases caused by_ Sclerotium rolfsii _and southern root-knot nematode_

by

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was accepted for publication in

_Plant Disease_

The American Phytopathological Society

St. Paul, MN

on 7 April 2010

and has been reprinted here with permission
ABSTRACT


Southern blight (*Sclerotium rolfsii*) and root-knot nematodes (*Meloidogyne* spp.) cause severe damage to fresh-market tomato (*Solanum lycopersicum*) throughout the southeastern US. Grafting is an emerging technology in US tomato production, and growers require information regarding the resistance characteristics conferred by rootstocks. In this study, southern blight (SB) and root-knot nematodes (RKN) were effectively managed using inter-specific hybrid rootstocks. During 2007 and 2008, field trials were carried out at two locations that had soils naturally-infested with *S. rolfsii*. At the end of the growing seasons, the mean SB incidence of non-grafted plants was 27% and 79% at the two sites. SB incidence among plants grafted onto rootstock cultivars 'Big Power' (one location only), 'Beaufort', and 'Maxifort' ranged from 0-5%, and AUDPC values were lower than non- and self-grafted controls (*P*<0.01). At one location, soils were naturally-infested with RKN and all three rootstocks reduced RKN AUDPC and RKN soil populations at first harvest (*P*<0.01). 'Big Power' was particularly effective at reducing RKN galling and RKN soil populations at final fruit harvest (*P*<0.01). Fruit yield was higher when resistant rootstocks were utilized (*P*<0.05), and in our study grafting was effective at maintaining crop productivity in soils infested with *S. rolfsii* and *M. incognita*. 
INTRODUCTION

Vegetable grafting is practiced worldwide (33) and gaining interest in the US for open-field and high tunnel tomato (*Solanum lycopersicum*) production (17). This technology has been primarily used for intensively-managed crops grown in greenhouses or tunnels. There are a variety of grafting techniques (18), but the most widely-adopted method worldwide for grafted tomato production is 'tube grafting' (26, 31). Resistant rootstocks are available for tomato, and can be used to manage economically important soilborne pathogens such as *Ralstonia solanacearum* (11, 27, 29, 38) and root-knot nematodes (*Meloidogyne* spp.) (1, 4).

Another serious disease on tomato in the Southeast US where grafting offers a promising management alternative is southern blight (SB) caused by *Sclerotium rolfsii*. SB affects fresh-market tomato throughout the southeastern US (22). *S. rolfsii* has a broad host range (>600 species), and over 270 susceptible host genera have been described (9). Diseases caused by *S. rolfsii* are most severe in the tropics and subtropics and in areas of the southern US where temperatures are sufficiently high to promote the growth and survival of the fungus (30). SB of tomato results in rapid and permanent wilt of all aboveground parts (22). As disease develops, mycelial growth covers the host tissue, and tan to reddish brown sclerotia form on the mycelial mat (30). Sclerotia serve as survival structures and can remain viable for multiple years (22, 30). Control of SB is difficult when inoculum levels are high and environmental conditions are suitable for disease development (22). Since *S. rolfsii* has a wide host range and can persist on many
types of crop residue, rotation to non-hosts is difficult (30). A physical barrier to protect the stem at the soil line is recommended for gardeners (22), but is probably not practical for commercial growers. Numerous fungicides and soil fumigants inhibit the germination of sclerotia or the mycelial growth of *S. rolfsii* (25, 30), but may be cost prohibitive in conventional systems and are not allowed in organic production.

Host resistance could be a suitable management strategy for SB, but breeders have had little success at identifying and deploying host resistance against *S. rolfsii* into commercial cultivars. Resistance to SB was first identified in *Lycopersicon pimpinellifolium* (24). Six SB-resistant tomato breeding lines were reported in 1992 (19), but to date, no known resistance occurs in available cultivars for fresh-market tomato production (22, 42).

Grafting has been utilized to manage root-knot nematodes (RKN) in tomato crops worldwide (1, 4, 6), but little work has been carried out in naturally-infested soils in the US (17, 21). RKN are obligate endoparasites that infect over 1,700 plant species and cause severe losses in crop yield (3, 28, 34, 40). Although >50 species of *Meloidogyne* have been described, four species, *M. incognita*, *M. arenaria*, *M. javanica*, and *M. hapla*, are considered the most economically important worldwide (40). In the US, *M. incognita* (southern root-knot nematode) is most prevalent on tomato, although *M. hapla* occurs in northern climates (28). Low populations of RKN may cause little economic damage on tomato, but severe infestations result in extensive root-galling, wilting, nutritional deficiencies, and stunting of the plant (28).
Nematicides can be cost-effective for managing diseases caused by *Meloidogyne* spp. in high-value vegetable crops (8). The soil fumigants methyl bromide, methyl iodide, and 1,3-dichloropropene have nematicidal properties (13), but methyl bromide is currently in the process of worldwide phase-out (8). Crop rotation with non-hosts is effective at reducing root galling among susceptible vegetable crops (16, 36). However, the wide host range of *M. incognita* makes development of profitable rotations difficult without the use of resistant crops.

Many commercial tomato cultivars carry a major resistance gene, *Mi*, that confers effective resistance against *M. incognita, M. arenaria*, and *M. javanica* (10, 40). This resistance was identified in an accession of *Lycopersicon peruvianum*, and the *Mi* gene has been introgressed into cultivated tomato (40). In *Mi*-mediated resistance, a hypersensitive response (HR) occurs resulting in programmed cell death and ultimately disruption of the nematode life cycle (40). This resistance mechanism is effective, but can be lost at soil temperatures above 28°C (7, 40). Research in Spain showed variable infection and reproduction rates of *M. javanica* in tomato among 10 rootstocks, all of which carried the *Mi* gene (6). Among the rootstocks tested, ‘Maxifort’ and ‘Beaufort’ were susceptible to infection by *M. javanica*. Similar results were seen in greenhouse studies with *M. incognita* (21) on ‘Beaufort’ rootstock, but fruit yield was not adversely affected by nematode damage suggesting host tolerance. The results of these studies indicate that *Mi* is not functioning in a manner that is typical of HR-mediated resistance in some inter-specific hybrid tomato rootstock cultivars including 'Maxifort' and
'Beaufort'. These are the most accessible rootstocks to growers in the US and 'Maxifort' was recommended for heirloom tomato production in a recent study (32). The ability of these rootstocks and others at reducing infection and crop losses by RKN in naturally-infested soils in the US is unknown.

This report determined the relevance of grafting as a viable mechanism for disease control and yield benefits in organic and conventional tomato production systems in North Carolina. There were three primary objectives: (i) to evaluate the utility of three rootstock hybrids, 'Beaufort', 'Maxifort', and 'Big Power' to reduce the incidence of SB caused by *S. rolfsii* in naturally-infested soils, (ii) to determine the efficacy of these rootstocks at reducing galling caused by native populations of root-knot nematode, and (iii) to ascertain how the deployment of resistant rootstock impacts fruit yield and whether it is a potentially useful IPM strategy against SB and RKN on tomato.
MATERIALS AND METHODS

Transplant production and grafting: All grafted and non-grafted tomato transplants were produced at the Southeastern Plant Environment Laboratory located at North Carolina State University (Raleigh, NC; http://www.ncsu.edu/phytotron/).

Commercially-available heirloom cultivars, ‘Cherokee Purple’ (Johnny’s Selected Seeds; Winslow, ME) and ‘German Johnson’ (Totally Tomatoes. Randolph, WI), were used as the scion cultivar for rootstock treatments and for non- and self-grafted controls. These open-pollinated cultivars are used regionally for fresh-market production, and no known resistance to SB or RKN has been reported. ‘Cherokee Purple’ and ‘German Johnson’ were used at the Alamance and Sampson County locations, respectively.

Commercially-available rootstock cultivars, 'Big Power' (Rijk Zwaan Seeds, De Lier, The Netherlands), 'Beaufort' (De Ruiter Seeds; Bergschenhoek, The Netherlands), and 'Maxifort' (De Ruiter Seeds; Bergschenhoek, The Netherlands), were used as rootstock treatments. These inter-specific rootstock hybrid cultivars are typically utilized for tunnel and greenhouse production throughout the U.S., Canada, Northern Europe, and the Mediterranean. All three rootstocks are *S. lycopersicum* L. × *S. habrochaites* (S. Knapp and D.M. Spooner), and are *Mi/Mi* homozygous resistant at the *Mi* locus (6).

In all trials, a self-grafted treatment was included where the scion variety was grafted onto its original root system to account for any grafting/healing effects. The Japanese tube grafting technique was carried out for all grafted plants (31). Rootstock
and scion seedling stems were severed and held together using a silicon clip. Once grafted, the plants were immediately moved into a "healing chamber" where humidity and light conditions are manipulated to promote graft union formation (See 31). Once grafting and subsequent healing had occurred, grafted and non-grafted tomato plants were transplanted to 10 cm pots and allowed to grow for 10-14 days in the greenhouse before being planted into field plots.

**Alamance County trials:** Field trials were conducted in 2007 and 2008 at a commercial organic farm located in Alamance County, NC (35.875959 N, 79.267759 W). Soil type consists of Efland silt loam (pH = 6.0). Organic practices have been employed for more than 20 years at this site. Heirloom tomatoes are rotated with cut flowers, non-solanaceous vegetables, and cover crops in 3-year intervals. Research trials were conducted within heirloom tomato production blocks in each year and located in multi-bay high tunnels. At this location, symptoms typical of RKN had not been seen in prior years, but SB was observed previously within pepper and tomato production blocks.

The grafting treatments included ‘Cherokee Purple’ grafted onto 'Beaufort', and 'Maxifort' rootstocks in addition to non-grafted and self-grafted ‘Cherokee Purple’. In both years, a randomized complete block design was utilized with four replications, and planted within two, 30 m rows (2 blocks per row). The four grafting treatments were randomly assigned to 3.8 m plots within each of the four blocks; treatments were re-randomized in 2008. Seven plants were located within each plot and cultural methods
were consistent with on-farm heirloom tomato production. Plants were spaced at 46 cm within the row, and rows were 1.52 m apart. Pre-plant nitrogen was supplied through cover crop incorporation and supplemental feathermeal applications of 55 and 111 Kg N/ha in 2007 and 2008, respectively, and water was provided by drip irrigation. Fabric mulch was used to suppress weeds, and a vertical trellis system was built using steel posts and 1.3 m wide wire mesh with 10 cm x 10 cm spacing. Vines were trained to the trellis with vinyl tape.

The Alamance County experiments were initiated on 5 May 2007, and 25 Apr 2008. In 2007, harvesting was carried out on: 26 and 29 Jun; 3, 9, 12, 17, 19, 23, 26, and 30 Jul; 2, 6, 14, 16, 20, 23, 27 and 30 Aug; 3, 6, 10, and 13 Sep. In 2008, harvesting was conducted on: 23, 26, and 30 Jun; 3, 8, 11, 14, 18, 21, 25, and 29 Jul; 1, 7, 12, 15, 18, 21, 25, and 28 Aug; 1 and 4 Sep. All tomato fruit were harvested and graded as marketable or non-marketable based upon on-farm standards, and fruit weight and number were recorded for each grade.

**Sampson County trials:** Field trials were conducted in 2007 and 2008 at the Horticultural Crops Research Station, located in Sampson County, NC (35.023244 N, 78.278146 W). The research trials were carried out in a field that is naturally-infested with a native population of southern RKN (*Meloidogyne incognita*). Soil type consisted of Norfolk loamy sand (pH = 5.7). This trial was treated in a conventional manner, and typical herbicide and insecticide applications were administered.
The grafting treatments included ‘German Johnson’ grafted onto ’Big Power’, 'Beaufort', and 'Maxifort' rootstocks as well as non- and self-grafted controls. In addition, a fumigant treatment was included whereby non-grafted plants were grown in plots that had been fumigated with 1,3-dichloropropene, a broad-spectrum nematicide (8). In both years, a randomized complete block design with four replications was utilized and treatments were re-randomized in 2008. The six treatments were randomly assigned to 7.6 m plots within each of the four, 56.1 m rows that served as blocks. An unplanted 1.5 m buffer was included between all plots to reduce interplot interference between fumigated and non-fumigated treatments. Cultural methods were consistent with open-field tomato production in the region. A raised-bed plasticulture system was employed using white plastic mulch and drip irrigation. Raised beds were 15 cm high and 75 cm wide. In-row spacing was 46 cm in 2007 and 61 cm in 2008. Stake-and-weave cultural management was used to train the plants vertically to 2 m metal posts located between every other plant.

Raised beds were formed and 1, 3-dichloropropene was applied to fumigated treatments on 24 May and 16 Apr in 2007 and 2008, respectively. Telone II (Dow AgroSciences, LLC; Indianapolis, IN) was applied at 136 kg/ha (broadcast) through two 20 cm shank injectors, spread 30 cm apart, as the beds were formed and covered with white plastic mulch. Grafted and non-grafted transplants were set in the field on 22 Jun 2007 and 15 May 2008. In 2007 and 2008, 14 and 12 plants of each treatment were centrally-located within the 7.6 m plot, respectively. Fruit was harvested on: 29 Aug; 5,
12, and 19 Sep in 2007; and 6, 12, 20, and 26 Aug in 2008. Final fruit harvest included all fruit larger than 5 cm. All fruit were harvested and graded as marketable or non-marketable based upon the appearance of fruit shriveling, sunscald, blossom-end rot, insect damage, or severe fruit cracking.

**Disease assessment:** SB disease ratings were performed non-destructively at weekly intervals. Disease incidence was recorded based on the presence of typical signs and symptoms of SB. Tomato plants that have been infected and killed by *S. rolfsii* undergo rapid and permanent wilt, and mycelium and sclerotia are visible on the stem. During the course of the season, stems of plants that had been killed by *S. rolfsii* were removed and incubated to verify the presence of viable sclerotia, and any remaining fruit was harvested and recorded.

Because the Sampson County location had a history of RKN infestation, root-knot nematode severity was assessed through biweekly destructive sampling and examination of the roots for galling and other damage caused by *Meloidogyne* spp. Destructive sampling was carried out on 25 Jul; 8 and 22 Aug; 5 and 19 Sep; and 1 Oct in 2007 and 17 Jun; 1, 15, and 29 Jul; 12 and 25 Sep in 2008. After the upper 30 cm of roots were removed from the soil, the galling index was rated using the system outlined by Zeck (41) on a 0-10 scale (0 = no observable galls, 1 = very few small galls, 2 = numerous small galls, 3 = numerous small galls of which some are grown together, 4 = numerous small and some big galls, 5 = 25% or roots severely galled, 6 = 50% of roots
severely galled, 7 = 75% of roots severely galled, 8 = no healthy roots but plant is still green, 9 = roots rotting and plant dying, 10 = plant and roots dead). In Alamance County, three root samples from all plots were visually examined for RKN damage at the end of the growing season in both years.

In Sampson County, soil samples were taken at first harvest and final harvest in both years to observe root-knot nematode soil populations. Soil was gathered using a nematode-specific sampling soil core that allows for 8-16 soil cores to be stored in the upper column of the sampler. Approximately 1 L of soil per plot was systematically sampled from the root zone by removing cores from the upper 10 cm of soil that were within 15 cm of the main stem. Bulked 1 L soil samples from each plot were thoroughly mixed and 500 cc of soil was analyzed by the Nematode Assay Section of the North Carolina Department of Agriculture and Consumer and Agronomic Services (Raleigh, NC; http://www.ncagr.gov/agronomi/nemhome.htm) for the presence of RKN juveniles. Plant parasitic nematodes were extracted from samples by a combination of elutriation (5) and centrifugation (15) and nematodes were identified by morphology (12). Soil was sampled from the field on 29 Aug and 19 Sep in 2007; and 6 and 26 Aug in 2008. A 1 L soil sample was also systematically taken from the entire field 6 Aug 2008 and submitted for identification of the dominant species within the field. Species identification was carried out through perineal patterns and host-differentiation (12). Sequence of the ITS1 region of DNA extracted from RKN recovered was also included in RKN species analysis.
**Statistical Analysis:** All data were analyzed using analysis of variance (PlotIt; Scientific Programming Enterprises, Haslett, MI), and where significant treatment effects were identified, a mean separation test was carried out using an F-protected least significant difference (LSD) test. Data from the two locations were treated in a similar manner, but were analyzed independently. Data collected in 2007 and 2008 were combined and analyzed using split-plot factorial ANOVA whereby main plots included grafting treatments and sub-plots represent the two years at a given location. The occurrence of statistical interaction between grafting and year determined the presentation of pooled data. LSD tests were carried out at $P = 0.05$ and $P = 0.01$. Total (both marketable and culled fruit) and marketable yield were analyzed in a similar manner and was calculated to represent tonnes/hectare (t/ha) in the tables. In the Sampson County trial, fumigation was utilized to compare grafting with a registered nematicidal fumigant and not included as a factorial element. Therefore, it was analyzed as a grafting effect treatment in the full grafting*year split-plot design. Disease progress curves for SB incidence and RKN galling index over time were plotted. An area under the disease progress curve (AUDPC) value was calculated (35) and analyzed using split-plot factorial ANOVA. Root knot nematode populations were log transformed ($\log_{10} (1+x)$) for analysis and separated means were back-transformed for presentation of the data.
RESULTS

SB (caused by *S. rolfsii*) was observed at both locations and during both years of the research trials. The mean incidence of SB among non- and self-grafted treatments ranged from 27-79% at the end of the growing season. Symptoms typical of RKN were seen in both years of the study in Sampson County, and the final RKN galling index of the non- and self-grafted plants was 9.9 and 9.3, respectively. The results of the differential host test and perineal pattern analysis indicated that the dominant species in the field was *Meloidogyne incognita* (race 1). ITS 1 DNA sequence from the nematodes in the field showed 100% similarity to *M. incognita* (AY438556.1), but also had high similarity to other *Meloidogyne* spp. (AY438554.1, AY438555.1, AY858795.1) (BLASTN Suite, National Center for Biotechnology Information, Bethesda, MD). No damage from RKN was observed at the Alamance County location in either year. A summary of the resulting P-values for each split-plot ANOVA are shown in Table 1.1. In all cases, grafting*year interactions were not significant at *P*=0.05. Therefore, data from 2007 and 2008 for each of the locations were combined and used for presentation.

**Alamance County:** The SB epidemic began among non- and self-grafted treatments 70 days after planting (DAP) and final disease incidence for non-grafted and self-grafted plants was 27% and 39%, respectively (Fig 1.1). Total and marketable fruit yield (t/ha) was significantly greater among treatments with 'Beaufort' and 'Maxifort' rootstock compared to non-grafted and self-grafted treatments (*P* = 0.01, Table 1.2) and no
significant differences in fruit yield were observed between the rootstocks. Total yield increased with the use of rootstock by 36-75%, and marketable yield increased by 34-79%. Average total and marketable fruit size was significantly larger with 'Beaufort' and 'Maxifort' rootstock ($P = 0.01$, Table 1.2), and total fruit number was higher among rootstock treatments ($P = 0.01$, Table 1.2).

**Sampson County:** SB incidence rapidly increased throughout the non- and self-grafted treatments, and final SB incidence was 79% and 72%, respectively (Fig 1.2A). Plants grafted onto 'Big Power', 'Beaufort', and 'Maxifort' showed low levels of SB (1-5%), and fumigated plots had 51% final disease incidence. The impact of the epidemic over time was significantly lower among rootstock treatments as indicated by the AUDPC values ($P = 0.01$, Fig 1.2B). Fumigation with Telone II reduced SB as compared to non-grafted plants, but had significantly higher AUDPC values than the rootstock treatments ($P = 0.01$, Fig 1.2B).

Damage from RKN was observed in both years (Fig 1.3), and non- and self-grafted treatments had severe galling (RKN index >5) 61 DAP (Fig 1.3A). No galling was seen prior to 61 DAP among the fumigated treatments, but resulted in a RKN index of 7.1 by final fruit harvest. 'Beaufort' and 'Maxifort' rootstock showed moderate galling throughout the season and the final RKN index was 2.8 and 2.9, respectively. The RKN index of 'Big Power' remained at zero until 90 DAP, and the final galling index was 0.25 (Fig 1.3A). The RKN index AUDPC values showed highly significant separation of the
means ($P = 0.01$, Fig 1.3B). Fumigation reduced the AUDPC values as compared to non- and self-grafted controls. However, 'Maxifort' and 'Beaufort' rootstock treatments showed lower AUDPC values compared to non-grafted, self-grafted, and fumigated treatments, and 'Big Power' AUDPC values were even less than 'Maxifort' ($P = 0.01$, Fig 1.3B).

At first harvest, extremely high populations of RKN (>8,000 nemas / 500 cc soil) were found among non-grafted and self-grafted treatments (Table 1.3). Fumigation with 1,3-dichloropropene significantly reduced populations at first harvest, but populations were not significantly lower than the controls at the final harvest at $P=0.01$ (Table 1.3) or $P=0.05$ (data not shown). 'Beaufort' and 'Maxifort' rootstock treatments significantly reduced populations as compared to non- and self-grafted treatments at first harvest, but were statistically similar to non-grafted, self-grafted, and fumigated treatments at final harvest. 'Big Power' had the lowest population at first harvest, and was the only treatment that significantly suppressed populations at final harvest ($P = 0.01$, Table 1.3).

Total and marketable yield was greater in 'Big Power', 'Beaufort', and 'Maxifort' rootstock treatments as compared to non-grafted, self-grafted and fumigated treatments ($P = 0.05$, Table 1.4). Fumigation increased total and marketable yield by 55% and 75% as compared to non-grafted treatments, but this difference was not statistically significant ($P = 0.05$, Table 1.4). 'Beaufort' showed the highest yield of all treatments but was not significantly different as compared to the other two rootstock
treatments ($P = 0.05, \text{Table 1.4}$). Total and marketable fruit yield was increased among rootstock treatments by 70-173% and 132-248%, respectively, compared to non- and self-grafted treatments.

Average total fruit size was highest among the three rootstock treatments ($P = 0.05, \text{Table 1.4}$), and 'Maxifort' had the largest total fruit size. 'Big Power' had the smallest total fruit size of the rootstock treatments, but was statistically similar to 'Beaufort' and 'Maxifort', and was larger than the non-grafted, self-grafted, and fumigated treatments ($P = 0.05, \text{Table 1.4}$). Fruit number was also impacted by rootstock and 'Beaufort' had the highest fruit number but was statistically similar to 'Big Power' and 'Maxifort' ($P = 0.05, \text{Table 1.4}$).
CONCLUSIONS

Grafting with resistant rootstocks could play an important role in management of \textit{S. rolfsii} and \textit{M. incognita} in US high tunnel and field tomato production. This technology can be quickly deployed without causing significant changes in farming operations and can aid in the development of successful crop rotation systems. Our results demonstrated that SB can be effectively managed through the use of the interspecific rootstocks. In the Alamance County trials, these rootstocks provided complete resistance to SB in both years. In Sampson County, SB was particularly severe and 'Big Power', 'Beaufort', and 'Maxifort' showed exceptionally high levels of disease resistance to SB. At this location, final disease incidence of the rootstocks ranged from 1-5%, suggesting that host resistance among these rootstocks is quantitative in nature.

\textit{Sclerotium rolfsii} produces large amounts of oxalic acid and other cell wall-degrading enzymes as it infects its host, and plant tissues tolerant of these enzymes may be resistant to hyphal invasion (30). Resistance to SB in tomato breeding lines was attributed to precocious development of secondary tissue on the basal mainstem (19). In other crops, resistance to infection by \textit{S. rolfsii} is related to calcium concentration within host tissue (30), and this relationship has been suggested in tomato (24, 39). Inter-specific rootstocks play an important role in macro- and micro-nutrient uptake and metabolism (33). However, many studies that address this area utilize leaf tissue analysis, distal from the infection court of \textit{S. rolfsii}. One report showed that the rootstock 'Beaufort' elevated calcium in the epigeous biomass (stem, leaf, and fruit
tissues) of grafted tomato plants (20). A productive area for future research would be to identify the genetic and mechanistic source of resistance to SB seen in this study.

In the Sampson County trials, the severity of SB among the non- and self-grafted plants could have been increased by root-knot nematode damage. 1,3-dichloropropene showed no efficacy against *S. rolfsii* when applied through drip application (23). In contrast, the shank application utilized in our study reduced SB AUDPC values compared to non-grafted controls. The difference seen between our study and others (23) may have been the result of decreased RKN damage, and therefore reduced susceptibility to SB. There is conflicting evidence in the literature on whether RKN damage can predispose tomato to increased SB susceptibility (23, 28, 30). However, regardless if predisposition is occurring, 'Beaufort' and 'Maxifort' sustained moderately-high levels of damage from RKN and these rootstock cultivars maintained effective resistance to SB. To our knowledge, this report represents the first evidence of effective use of host resistance against SB in tomato among commercially-available cultivars, and grafting technology provides a unique opportunity to quickly deploy this resistance into established production systems.

At the Sampson County location grafting with resistant rootstocks reduced galling and reproduction by RKN in naturally-infested soils. 'Big Power' had higher levels of resistance to RKN than 'Beaufort' and 'Maxifort' throughout the growing season and the results of this study suggest that this rootstock is more effective at reducing RKN populations in infested soils than fumigation. 'Maxifort' and 'Beaufort'
displayed partial resistance to RKN compared to the non- and self-grafted controls as indicated by the AUDPC. 'Big Power' had high resistance in soils naturally-infested with *M. javanica* (6), and our study showed similar results with *M. incognita*. The low RKN index and corresponding AUDPC values displayed by plants grafted with 'Big Power' rootstock indicate that resistance in this rootstock is effective at reducing infection and establishment of feeding sites. There was slight galling seen on 'Big Power' at the end of the season, but this loss-of-function and its relation to heat stability is not clear. In this study, the heirloom cultivar 'German Johnson' was utilized as a susceptible control. Future studies that evaluate the efficacy of *Mi* in rootstocks would benefit from a non-grafted or rootstock treatment that utilizes a commercial hybrid cultivar containing the functional *Mi* gene.

In the rootstocks utilized for this study, the *Mi* gene was previously verified by REX-1 and Mi23 molecular markers (6). Recent studies indicate that host resistance in inter-specific rootstocks containing *Mi* have variable efficacy at reducing infection and reproduction by *Meloidogyne javanica* (6) and *M. incognita* (21). Our results found that *M. incognita* can infect and reproduce on 'Beaufort' and 'Maxifort' rootstocks. Similarly to the non- and self-grafted plants, galling was first observed on 'Maxifort' and 'Beaufort' rootstocks 47 DAP suggesting that heat stability did not play a significant role in the efficacy of *Mi*. Other studies (6) also indicate that susceptibility of these rootstocks to RKN is not related to soil temperature. However, due to the unexpected results in regards to RKN shown by 'Beaufort' and 'Maxifort' in this study and others, it
appears that further field evaluations of inter-specific rootstocks in naturally-infested soils are needed to provide rootstock recommendations in the southeastern US.

At the Sampson County location, all three rootstocks maintained fruit production under exceptionally high levels of disease pressure from RKN and SB. 'Beaufort' and 'Maxifort' had the highest yields, even though RKN populations were moderately high at first harvest. In previous greenhouse studies with 'Beaufort' (21), the authors suggested that this rootstock confers tolerance to RKN and our results were similar. However, because SB and RKN were both present at this location, it's difficult to determine if tolerance is being conferred by 'Beaufort' and 'Maxifort' rootstocks. Similarly, the lack of statistical separation for fruit yield between fumigated and non-fumigated plots could be the result of the combined impact of SB and the rapid increase in RKN damage seen late in the season.

Fruit marketability was higher among the three rootstocks in the Sampson County trials. Fruit marketability can be affected by SB as severe wilting leads to fruit shriveling and sunscald. Although the specific parameters of non-marketable fruit were not recorded, resistance to SB among rootstock treatments may have increased the likelihood of marketable fruit in the Sampson County trials. Although disease pressure was less severe, fruit yield of grafted plants was higher at the Alamance County location as well. Rootstocks may also provide elevated yield through added vigor and plant growth (17, 33), but recent trials in NC with 'German Johnson' did not show consistent
yield effects of grafting with 'Maxifort' rootstock under little or no disease pressure (32).

Host resistance is a preventative IPM strategy that is used to avoid disease outbreaks in the field (14). In this study, 'Big Power' was more effective than fumigation at reducing RKN populations at the end of the growing season. This finding may be important for growers who double-crop or follow tomatoes with susceptible cash crops. Resistant pepper cultivars have been used to manage root-knot nematode damage in susceptible cucurbit crops grown in double-cropping production systems (37). Our data suggests that grafting with 'Big Power' offers flexibility as growers develop profitable rotation programs. Similarly, sclerotia formation by *S. rolfsii* positively correlates with SB disease incidence and severity in following years (30).

Grafting will be an important component for US tomato growers that are seeking non-chemical strategies to manage soilborne diseases. The resistance to SB seen here is an example of how grafting with inter-specific rootstocks provides a platform for host resistance deployment where major resistance genes are not available for commercial cultivars. Quantitative resistance traits are often widely-known among wild species, but their deployment into commercial cultivars can be problematic. Resistance to SB was first identified in 1959 (24) and successful incorporation of resistance into commercial cultivars for fresh-market production has not been accomplished to date (22, 42). Similarly, alternative sources of resistance to *Meloidogyne* spp. have been identified in wild accessions of tomato (i.e. heat-tolerance and resistance to *M. hapla*) even though
their contribution to cultivated tomato production has been insignificant thus far (2, 40). The benefit of grafting with inter-specific rootstock is that breeding programs can readily-utilize host resistance from wild plant species without penalizing fruit production, and this technology may be a vital component in an IPM program that seeks to reduce damage caused by soilborne pathogens without the use of chemical fumigants.
Table 1.1. Effects of year and grafting for Alamance and Sampson County tomato trials

<table>
<thead>
<tr>
<th>Location</th>
<th>Parameter</th>
<th>Year</th>
<th>Grafting</th>
<th>Year*Grafting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alamance Co.</td>
<td>Southern blight AUDPC&lt;sup&gt;x&lt;/sup&gt;</td>
<td>0.015</td>
<td>&lt;0.001</td>
<td>0.095</td>
</tr>
<tr>
<td></td>
<td>Total yield (t/ha)</td>
<td>0.016</td>
<td>&lt;0.001</td>
<td>0.250</td>
</tr>
<tr>
<td></td>
<td>Total fruit number</td>
<td>0.026</td>
<td>&lt;0.001</td>
<td>0.406</td>
</tr>
<tr>
<td></td>
<td>Total fruit size</td>
<td>0.012</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marketable yield (t/ha)</td>
<td>0.008</td>
<td>&lt;0.001</td>
<td>0.117</td>
</tr>
<tr>
<td></td>
<td>Marketable fruit number</td>
<td>0.017</td>
<td>&lt;0.001</td>
<td>0.150</td>
</tr>
<tr>
<td></td>
<td>Marketable fruit size</td>
<td>0.004</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percent marketable (by weight)</td>
<td>0.020</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percent marketable (by number)</td>
<td>0.116</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sampson Co.</td>
<td>Southern blight AUDPC&lt;sup&gt;x&lt;/sup&gt;</td>
<td>0.247</td>
<td>&lt;0.001</td>
<td>0.101</td>
</tr>
<tr>
<td></td>
<td>Root-knot AUDPC&lt;sup&gt;x&lt;/sup&gt;</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Root-knot pop. (first harvest)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.080</td>
<td>&lt;0.001</td>
<td>0.402</td>
</tr>
<tr>
<td></td>
<td>Root-knot pop. (final harvest)&lt;sup&gt;z&lt;/sup&gt;</td>
<td>0.010</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total yield (t/ha)</td>
<td>0.075</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total fruit number</td>
<td>0.170</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total fruit size</td>
<td>0.019</td>
<td>&lt;0.001</td>
<td>0.346</td>
</tr>
<tr>
<td></td>
<td>Marketable yield (t/ha)</td>
<td>0.208</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marketable fruit number</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Marketable fruit size</td>
<td>0.003</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percent marketable (by weight)</td>
<td>0.170</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Percent marketable (by number)</td>
<td>0.161</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

<sup>w</sup> Data from 2007 and 2008 were pooled and analyzed using split-plot ANOVA. Blank spaces denote F-value <1.

<sup>x</sup> Calculated area under the disease progress curve (AUDPC) values.

<sup>y</sup> Root-knot nematode soil populations sampled on the first day of fruit harvest.

<sup>z</sup> Root-knot nematode soil populations sampled on the last day of fruit harvest.
### Table 1.2. Combined tomato fruit yield and marketability of grafted and non-grafted 'Cherokee Purple at Alamance County'

<table>
<thead>
<tr>
<th></th>
<th>Marketable fruit yield</th>
<th></th>
<th>Total fruit yield</th>
<th></th>
<th>% Marketability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(t/ha)</td>
<td>Size (g)</td>
<td>No. (10^3/ha)</td>
<td>(t/ha)</td>
<td>Size (g)</td>
</tr>
<tr>
<td>Non-grafted</td>
<td>56.1 a</td>
<td>253 a</td>
<td>221.3 ab</td>
<td>75.6 a</td>
<td>244 a</td>
</tr>
<tr>
<td>Self-grafted</td>
<td>45.5 a</td>
<td>249 a</td>
<td>183.1 a</td>
<td>64.7 a</td>
<td>238 a</td>
</tr>
<tr>
<td>Beaufort</td>
<td>75.0 b</td>
<td>306 b</td>
<td>245.4 bc</td>
<td>102.7 b</td>
<td>289 b</td>
</tr>
</tbody>
</table>
| Maxifort       | 81.4 b | 300 b    | 271.6 c       | 112.9 b | 291 b    | 388.0 b        | 71.3 a  | 69.4 a  

* Pooled yield data from 2007 and 2008. Values followed by the same letter are not significantly different according to a protected least significant difference test (P= 0.01)
* Under moderate disease pressure from southern blight (S. rofisii).
* Inter-specific rootstocks were grafted with 'Cherokee Purple' scion.

### Table 1.3. Root-knot nematode soil population at Sampson County

<table>
<thead>
<tr>
<th></th>
<th>First harvest</th>
<th>Final harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-grafted</td>
<td>8357 d</td>
<td>1964 b</td>
</tr>
<tr>
<td>Self-grafted</td>
<td>8751 d</td>
<td>1220 b</td>
</tr>
<tr>
<td>Telone II*</td>
<td>379 b</td>
<td>1260 b</td>
</tr>
<tr>
<td>Big Power*</td>
<td>77 a</td>
<td>40 a</td>
</tr>
<tr>
<td>Beaufort*</td>
<td>2680 c</td>
<td>2542 b</td>
</tr>
<tr>
<td>Maxifort*</td>
<td>3091 c</td>
<td>1251 b</td>
</tr>
</tbody>
</table>

* Values represent numbers of root-knot nematode juveniles per 500 cc soil.
* Pooled yield data from 2007 and 2008. Values followed by the same letter are not significantly different according to a protected least significant difference test (P= 0.01)
* Root-knot nematode soil populations sampled on the first day of fruit harvest
* Root-knot nematode soil populations sampled on the last day of fruit harvest
* Non-grafted 'German Johnson' grown in plots fumigated with Telone II (1.3-D)
* Inter-specific rootstocks were grafted with 'German Johnson' scion.
<table>
<thead>
<tr>
<th></th>
<th>Marketable fruit yield</th>
<th>Total fruit yield</th>
<th>% Marketability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(t/ha)</td>
<td>Size (g)</td>
<td>No. (10^3/ha)</td>
</tr>
<tr>
<td>Non-grafted</td>
<td>10.5 a</td>
<td>250 a</td>
<td>41.8 a</td>
</tr>
<tr>
<td>Self-grafted</td>
<td>12.9 a</td>
<td>256 a</td>
<td>50.3 a</td>
</tr>
<tr>
<td>Telone II^7</td>
<td>18.4 a</td>
<td>279 ab</td>
<td>65.9 ab</td>
</tr>
<tr>
<td>Big Power^8</td>
<td>30.1 b</td>
<td>283 abc</td>
<td>106.4 c</td>
</tr>
<tr>
<td>Beaufort^2</td>
<td>36.3 b</td>
<td>295 bc</td>
<td>123.1 c</td>
</tr>
<tr>
<td>Maxifort^2</td>
<td>29.8 b</td>
<td>318 c</td>
<td>93.8 bc</td>
</tr>
</tbody>
</table>

^7 Pooled yield data from 2007 and 2008. Values followed by the same letter are not significantly different according to a protected least significant difference test (P≤ 0.05)

^8 Under severe disease pressure from southern blight (S. rolfsii) and root-knot nematodes (M. incognita).

^9 Non-grafted 'German Johnson' grown in plots fumigated with Telone II (1,3-Dichloropropene)

^2 Inter-specific rootstocks were grafted with 'German Johnson' scion.
Figure 1.1 – Mean southern blight incidence on non- and self-grafted ‘Cherokee Purple’ tomato as well as ‘Cherokee Purple’ grafted onto ‘Beaufort’ and ‘Maxifort’ rootstocks at the Alamance County location in 2007 and 2008.
Figure 1.2 – Mean southern blight incidence (A) and AUDPC (B) of non- and self-grafted ‘German Johnson’ tomato as well as ‘German Johnson’ grafted onto ‘Big Power’, ‘Beaufort’, and ‘Maxifort’ rootstocks and non-grafted plants grown in soil fumigated with Telone II (1,3-Dichloropropene). Data were collected from the Sampson County trials in 2007 and 2008 and AUDPC data were analyzed by separation of the means with a protected least significant difference test ($P = 0.01$).
**Figure 1.3** – Mean root-knot nematode galling index (A) and AUDPC values (B) of non- and self-grafted ‘German Johnson’ tomato as well as ‘German Johnson’ grafted onto ‘Big Power’, ‘Beaufort’, and ‘Maxifort’ rootstocks and non-grafted plants grown in soil fumigated with Telone II (1,3-Dichloropropene). Data were collected from the Sampson County trials in 2007 and 2008 and AUDPC data were analyzed by separation of the means with a protected least significant difference test ($P = 0.01$).
LITERATURE CITED


Due to the phaseout of methyl bromide and the expanding market for organic produce, tomato growers in the US require disease management strategies that reduce reliance on soil fumigation. The primary benefit of grafting is that scions can be selected based on local markets while rootstocks are utilized to manage site-specific soilborne pathogens. Inter-specific tomato rootstocks have been developed that are hybrids of cultivated tomato and a related wild species, but little is known regarding their efficacy against soilborne pathogens in the US. In this study, ‘Big Power’, ‘Beaufort’, and ‘Maxifort’ rootstocks showed excellent resistance to SB, caused by *S. rolfsii*. There is no known resistance to SB among commercial tomato cultivars, and this is a valuable finding for growers trying to manage this devastating pathogen. This study also highlights the importance of rootstock selection for growers that manage RKN. ‘Beaufort’ and ‘Maxifort’ had the highest yield, but allowed moderate RKN infection throughout the season. ‘Big Power’ was highly resistant to RKN and was more effective than fumigation at reducing RKN soil populations. Yield was significantly higher among grafted plants in all trials, and our data suggests that grafting offers flexibility as growers develop profitable rotation programs.
ACKNOWLEDGEMENTS

The authors wish to sincerely thank the NCSU Phytotron staff as well as Jim Driver, Mike Carnes, Amanda McWhirt, Ryan Faulk, and Seth Avis for technical assistance. We thank H.D. Shew and E.L. Davis for their excellent comments and special thanks to Dr. Weimin Ye and the NCDA Nematode Assay Section as well as our on-farm collaborators: Alex and Betsy Hitt, and the NCDA staff at the Horticultural Crops Research Station (Clinton, NC). Rootstock seed donated by De Ruiter Seeds and Rijk Zwaan Seeds. Funding provided by Southern Region SARE LS06-193 and USDA-NIFA 2007-51106-03794.
CHAPTER TWO

The following report

An economic analysis of two grafted tomato transplant production systems in the U.S.

by

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was accepted for publication in

HortTechnology
The American Society for Horticultural Science
Alexandria, VA

on 5 May 2010

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ABSTRACT

Grafting of herbaceous vegetables is an emerging development in the U.S. This report provides an estimate of the variable costs of grafting within U.S. tomato (*Solanum lycopersicum*) transplant production systems. Grafted and nongrafted plants were propagated at two commercial farming operations in North Carolina (NC) and Pennsylvania (PA) and the farm in NC produced certified organic transplants. Detailed economic production sequences were generated for each site, and grafted and nongrafted transplant production costs were $0.59 and $0.13 in NC, and $1.25, and $0.51 in PA, respectively. Direct costs associated with grafting (e.g. grafting labor, clips, chamber, etc) accounted for 37% to 38% of the added cost of grafting, and grafting labor was 11.1% to 14.4% of the cost of grafted transplant production. Seed costs represented 52% and 33% of the added cost of grafting at the two sites, and indirect costs (e.g. soil, trays, and heating) accounted for 10% and 30% of the added cost of grafting. Our findings suggest that under current seed prices and with similar production practices, the feasibility of grafting in the U.S. is not disproportionately affected by domestic labor costs. Additionally, the economic models presented in this report identify the cost of production at various transplant stages, and provide a valuable tool for growers interested in grafted tomato transplant production and utilization.
INTRODUCTION

Recently, grafting has gained increased attention for U.S. tomato production systems (King et al., 2008; Kubota et al., 2008). Field trials in the southeastern U.S. showed that resistant rootstocks can be utilized to manage site-specific soilborne diseases such as bacterial wilt (*Ralstonia solanacearum*) (Freeman et al., 2009; Rivard et al., 2008a; Rivard and Louws, 2008), fusarium wilt (*F. oxysporum* f.sp. *lycopersici*) (Rivard and Louws, 2008), southern blight (*Sclerotium rolfsii*) (Rivard et al., 2010), and root-knot nematodes (*Meloidogyne* spp.) (Kokalis-Burelle et al., 2008; Rivard et al., 2010). Furthermore, grafting has been proposed worldwide as a component in integrated pest management programs that are not dependent on the soil fumigant, methyl bromide (Besri, 2003, 2007; Minuto and Causarano, 2008). Methyl bromide has been an integral part of tomato production in the U.S., but is being phased out according to the Montreal Protocol (Martin, 2003; Noling and Becker, 1994; Sydorovych et al., 2008).

With the exception of the hydroponic greenhouse industry, few tomato growers in the U.S. utilize grafted transplants for fruit production (Kubota et al., 2008). There are two primary assumptions used to justify the lack of grafting in the U.S. The first is that U.S. labor costs are too high for manual grafting to be a feasible component of transplant production. Second, U.S. tomato fruit production systems may not generate the necessary per plant revenue to use grafted plants in a profitable way. Due to the recent regulatory issues associated with soil fumigants and the shift toward more-
intensive production systems among the organic sector, tomato growers are interested in producing grafted plants grown on the farm or purchased from specialized propagation facilities. Tomato grafting may provide an emerging niche for commercial propagators wishing to capture retail or wholesale markets, including organic and heirloom tomato plant sales (Rivard and Louws, 2006).

The most common tomato grafting technique for commercial propagation worldwide is tube grafting (Oda, 1999; Rivard and Louws, 2006). This technique is highly effective and can be performed when the plants are very small, thereby increasing throughput (Lee, 2003). The tube grafting procedure is relatively quick, and manual grafting rates range from 300-500 plants per hour (Kubota et al., 2008).

Even with the advent of tube grafting, legitimate concerns in the U.S. have been raised regarding the cost of labor required for the grafting procedure (Kubota et al., 2008). Grafting robots have been proposed as one alternative to reduce the cost of grafting labor worldwide (Lee, 2003), but their current use for tomato grafting is relatively low (Kubota et al., 2008).

In addition to manual grafting labor, grafted production requires other direct costs such as grafting supplies and healing chamber materials (Rivard and Louws, 2006). Additionally, rootstock seed must be purchased and excess seed must be planted (over-sowing) to account for less than 100% grafting success. Grafting also increases variable costs indirectly as it adds ~7 d onto the transplant production cycle and requires a complementary seedling crop of scion material prior to grafting (Rivard and
Louws, 2006). This increases materials, labor, and heating costs prior to grafting of the rootstock and scion seedling crops.

Although interest in grafting is high among U.S. growers and researchers, little work has been done to determine the economic costs and potential benefits of grafted transplant production for retail and commercial sale, or on-farm use. Furthermore, the first step in development of tomato production budgets with and without the use of grafting is to determine the cost of comparable grafted and nongrafted plants. Therefore, it is critical to identify the specific cost or range of costs that growers may experience as they produce grafted plants or implement grafting into their farming operations.

Published reports on the cost of grafted tomato transplants are rare. In Morocco, nongrafted and grafted tomato plants were produced for $0.19 and $0.38, respectively (Besri, 2003). In a recent review article, the use of grafted plants was estimated to add $0.60-$0.90 per transplant (excluding seed costs) for U.S. production (Kubota et al., 2008). Currently, about 40 million grafted transplants are imported into the U.S. for large greenhouse operations (Kubota et al., 2008), and the cost of the Canadian transplants is $2.00 per plant in addition to seed costs (C. Powell, personal communication). A preliminary report from NC suggested that the utilization of grafted plants would increase fruit production costs by $2275 per acre (O’Connell et al., 2009b), and a more in-depth description of transplant production methods as well as the subsequent analysis of the variable costs of production is provided here.
In order to identify the relevance of grafting for domestic tomato production, it is essential to determine the cost of grafted transplants propagated in the U.S.

Therefore, the goal of this study was: 1) to identify growers in the U.S. who successfully produced grafted tomato transplants, report their production models and any corresponding variable costs associated with grafted and nongrafted propagation; 2) to dissect the variable costs of grafted production and examine grafting labor and other key components that could be important considerations for propagation in the U.S.; and 3) to determine if tomato grafting can provide an economic benefit to local propagation facilities by adding value in tomato transplant production.
MATERIALS AND METHODS

Grafted and nongrafted tomato plants were produced at Black River Organic Farm (Ivanhoe, NC) and Good Harvest Farms (Strasburg, PA). Rootstock and scion cultivars were chosen based on the needs of each individual farm and market with grafting and healing chamber management conducted by farm personnel (Rivard and Louws, 2006). Three batches of grafted plants were produced throughout the spring at each site. Detailed transplant production sequences were formulated independently based on the experiences of each on-farm collaborator, and the variable costs associated with grafted and nongrafted transplant production were calculated. The specific transplant production methods varied at each location and are described below.

ON-FARM GRAFTING: NORTH CAROLINA SITE

In 2008, approximately 1000 certified organic grafted transplants were produced at Black River Organic Farm (Ivanhoe, NC) to determine the feasibility and utility of grafting for small-scale organic farming operation (O’Connell et al., 2009b). Transplants were produced in three batches of ~350 plants (3 to 5 true leaf stage). Cultivar Celebrity (Harris Seeds, Rochester, NY) was grafted as scion onto ‘RST-04-105-T’ (DP Seeds, Yuma, AZ) rootstock to manage southern bacterial wilt, caused by *R. solanacearum* (Rivard et al., 2008). In this case, the grower was producing grafted plants solely for use on the farm and transplants were produced in a greenhouse that
was utilized in the late spring and summer for vegetable production (Figs. 2.1 A-B). Therefore, heating costs were low and no mark-up was included in the final production cost. Grafted transplants were produced in accordance with organic certification. Organic fertilizers were used, potting media was listed by the Organic Materials Review Institute (OMRI), and rootstock and scion seed were untreated.

Rootstock and scion seeds were germinated in 512-cell plug trays within a small, heated germination chamber. After 7 d, rootstock and scion seedlings (1 true leaf stage) were transplanted into 50-cell trays (4.5 cm cell diameter) and allowed to grow for 14 d. Grafting was carried out when the plants had 2 to 3 true leaves. Once grafting had occurred, the plants were placed into a healing chamber (30” x 48”) constructed from rebar, concrete blocks, plastic sheeting, and a cool-mist vaporizer (Fig. 2.1A). The vaporizers were refilled twice daily and the chamber was managed for optimum healing conditions (Rivard and Louws, 2006). After the plants spent 7 d in the healing chamber, they were moved back into the greenhouse environment for 7 d to re-acclimate and finish healing prior to field planting (3 to 5 true leaf stage).

ON-FARM GRAFTING: PENNSYLVANIA SITE

In 2009, approximately 10,000 grafted transplants were produced at Good Harvest Farms (Strasburg, PA) to investigate the use of grafted tomato plants for high tunnel tomato production at Cedar Meadow Farm (Holtwood, PA) (Groff 2009a, 2009b). Good Harvest Farms houses a 40,000 ft² (3716 m²) transplant production facility for
local commercial and retail ornamental and vegetable plant sales. Grafted tomato transplants were produced in three batches of ~3300 grafted plants. Cultivar BHN 589 (BHN Seed, Immokalee, FL) was grafted as scion onto ‘Maxifort’ (De Ruiter Seeds, Bergschenhoek, The Netherlands) rootstock. In this case, the client was utilizing decreased planting densities for fruit production and requested that the plants were pruned into a “twin-leader” and grown in larger 18-cell trays (8 x 8 cm cell; Fig. 2.1D). Because the grafted plants at the PA location were being sold to a local tomato grower, a wholesale mark-up (50%) was included at the end of the economic production sequence.

Rootstock and scion seedlings were germinated by a local custom plug producer (York, PA) in 288-cell trays and allowed to grow for 3 d upon arrival at Good Harvest Farms. The seedlings (1 true leaf stage) were transplanted into 50-cell trays and allowed to grow for 21 d in the greenhouse before being grafted (2 to 3 true leaf stage). Once grafted, the plants were moved into a healing chamber for 7 d. The healing chamber was built on top of an “ebb-and-flow” greenhouse bench using wire hoops, plastic sheeting, shade fabric, and four cool-mist vaporizers (Fig. 2.1C). Similar to the other location, the grafted plants were monitored twice daily and light and humidity levels were maintained in accordance with current grafting protocols (Rivard and Louws, 2006). Once the plants (3 to 5 leaf stage) were moved back into the greenhouse, they were transplanted into an 18-cell tray, pruned, and grown for 14 d before being hardened off in an unheated greenhouse for 7 d prior to sale (Fig 2.1D). At the time of
sale, the plants had two leaders, and each leader had 3-5 true leaves (6-10 true leaf stage).

METHODOLOGY OF ECONOMIC ANALYSIS

A detailed grafted and nongrafted production sequence was generated for each location based on the data and experiences gained at each facility. Line items included all variable costs associated with grafted and nongrafted transplant production, and were specific to each location. Material prices used for transplant production and grafting are shown in Tables 2.1 and 2.2, and were used during the calculation of the variable production costs. Summaries of the variable per plant production costs are shown in Figures 2.2 A-B with the accumulation of variable production costs plotted on the y-axis. The discrete and continuous costs of each production stage are illustrated in the figures by the height of the rectangles and the slopes of the corresponding triangles, respectively (See Table 2.3). The transplant production sequence was plotted along the x-axis to illustrate the costs of specific transplant stages. A categorical summary of the variable costs for each location is shown in Tables 2.4-5, and the proportional cost of categorical line items in relation to the total cost of transplant production is shown in Table 2.6 (ITEM ÷ TOTAL). The distribution of the added costs of grafting is shown in Figure 2.3, and this approach describes the proportion of each factor (e.g. seed costs) in relation to the added cost of grafting [(SEEDgraft − SEEDnon) ÷ (TOTALgraft − TOTALnon)].
ASSUMPTIONS OF THE ECONOMIC MODEL

The production sequences did not consider fixed costs such as land value and initial costs of greenhouse structure. The objectives of the study were to determine the variable costs of grafted versus nongrafted transplant propagation and to ascertain any economic costs and benefits of integrating grafting in an existing tomato propagation facility.

Two base wage rates were utilized in the development of the budgets. It was assumed that $10.08/h was paid to hired labor and $14.00/h was paid to skilled/managerial labor at both locations (U.S. Department of Agriculture, 2009), but rates of $11.79 to $16.39/hr were used to account for workers’ compensation, unemployment, FICA taxes, and other indirect costs in addition to the base wage rate. Reusable materials such as the healing chamber and cool-mist vaporizers were depreciated according to the number of batches of grafted plants that could be produced (Tables 2.1-2). Similarly, variable costs incurred for healing chamber materials and construction correspond to the size of the batches that were grafted at each location (Tables 2.1-2).

Grafting clips were not reused and rootstock and scion seed costs were based on current market prices at the time of budget development. All plant and seed numbers were calculated to reflect plant and seed loss that may occur during germination, propagation, and grafting. Rootstock and scion seed were over-sown by 20% to account for germination rate and uniformity, and grafting success was assumed to be 90%,
consistent with grower experience. Grafting speed was 200 and 100 plants per person per hour at the NC and PA locations, respectively, and grafting labor was paid $10.08/h in NC and $14.00/h in PA.

At the PA location grafted plants were sold to a local tomato grower. However, no market had been established for locally-raised grafted transplants and so the imposed price was based on typical wholesale mark-up for commercial vegetable plant sales at Good Harvest Farms. The collaborating propagator indicated that a 50% mark-up was their typical “goal” and this value was used to calculate a selling price.
RESULTS

COST OF NONGRAFTED TRANSPLANT PRODUCTION

The total cost of nongrafted plants (TOTAL\textsubscript{non}) at the NC and PA locations was $0.13 and $0.51 per plant, respectively (Figs. 2.2A-B). At both locations, material costs (excluding seeds) comprised the highest expense, and ranged from 44.0% to 48.8% (Table 2.6). During production of nongrafted plants, labor costs ranged from 28.8% to 36.9% (Table 2.6). Although nongrafted seed prices were higher at the PA location than in NC (Tables 2.1-2), the overall seed costs were 14.3% of the total transplant costs in PA compared to 27.1% in NC (Table 2.6). Labor and materials added $0.10 per transplant at the NC site and $0.42 per plant on top of initial seed costs (Figs. 2.2A-B). Continuous costs (Table 2.3) accumulated more quickly in the PA production sequence (Fig. 2.2B) as compared to the NC site (Fig. 2.2A) and this is illustrated by the increased slope of the triangles on the economic timelines. The PA facility utilized both hourly and skilled labor during daily transplant care and watering and heating costs were higher than in NC (Tables 2.4-5).

Across the two locations, there was distinct difference in plant size that was required for use/sale. The transplant production methods required to produce the transplants in NC (3 to 5 true leaf stage) compared to the ones in PA (twin-leader, 6 to 10 true leaf stage) had a strong influence on variable costs. At the PA site, plants were grown for 2½ weeks longer than in NC, and were transplanted into an 18-cell tray, 3 weeks prior to sale (Figs. 2.2A-B). This production stage added transplanting labor and
materials and also substantial heating costs as each plant in an 18-cell tray required approximately three times as much space in the greenhouse as one in a 50-cell tray. The 18-cell production stage in PA added $0.23 per plant (Fig. 2.2B) which accounts for the majority of the per plant difference between the cost of nongrafted production at the two locations.

ADDITIONAL COSTS OF GRAFTED TRANPLANT PRODUCTION

The additional cost of producing a grafted plant ($\text{TOTAL}_{\text{graft}} - \text{TOTAL}_{\text{non}}$) was $0.46 and $0.74 per plant at the NC and PA sites, respectively (Table 2.4-5). These costs reflect additional rootstock and scion seed costs, the direct costs of grafting (e.g. grafting labor, clips, healing chamber, etc.), and the indirect costs associated with growing both a rootstock and scion crop prior to grafting. At the NC location, finished transplants had 3 to 5 true leaves, and additional seed costs (52%), grafting materials (24%), and grafting labor (14%) were the three most important variable expenses related to grafted transplant production (Fig. 2.3). At the PA location, finished transplants had 6 to 10 true leaves, and seed costs (33%), combined indirect costs (30%), and grafting labor (24%) were the three most important variable expenses in relation to grafting (Fig. 2.3).
SEED COSTS ASSOCIATED WITH GRAFTING

At both locations, the additional seed costs required for grafted transplant production were the most important single component in relation to the added costs of grafting (Fig. 2.3). The proportional seed costs of grafted plants accounted for 46.5% and 25.6% of the total cost of production at the NC and PA facilities, respectively, and were nearly double those of nongrafted plants at both locations (Table 2.6). The rootstock seed used in this study is relatively expensive ($0.17-$0.20/seed) compared to the prices of complementary scion seed or seed for nongrafts ($0.03-$0.07/seed). Currently, very few rootstock cultivars are available to U.S. growers, and the cost of rootstock seed could go down if a market develops in the U.S. Over-seeding and the assumed 90% grafting success had negligible impacts on scion seed costs but further amplified the increased price of rootstock (Tables 2.4-5).

DIRECT COSTS ASSOCIATED WITH GRAFTING

The direct costs of grafting include line items that were added into the production sequence of the grafted plants, but were not listed in the nongrafted budget and these costs comprised 37% to 38% of the additional costs of grafting at the two locations (Fig. 2.3). The “grafting / healing stage” added $0.18 and $0.31 per plant at the NC and PA sites, respectively (Figs. 2.2A-B).

Grafting labor accounted for 14% to 24% of the added cost of grafting (Fig. 2.3) and represented 11.1% to 14.4% of the total cost of production (Table 2.6). Per plant
labor costs are dependent on grafting speed, success, and the hourly wage of trained employees. In PA, a more conservative speed (100 plants/h) combined with the higher pay rate ($14/h) increased the per plant costs of manual grafting compared to the NC study. It is estimated that an experienced worker could graft >250 plants/h with >95% success (C.L. Rivard and F.J. Louws, unpublished data), and others have reported manual grafting rates from 300-500 plants/h for tube grafting (Kubota et al., 2008).

Grafting clips and healing chamber materials accounted for 24% and 13% of the added cost of grafting at the NC and PA sites, respectively (Fig. 2.3). The price of the silicone grafting clips ranged from $0.04 to $0.07 per clip (Tables 2.1-2). Grafting clips may be collected, sterilized, and re-used (Rivard and Louws, 2006), but the cost-effectiveness of this practice is unknown. Healing chamber materials and labor represented a relatively small portion (3.7% to 5.1%) of the total cost of grafted transplant production (Table 2.6). Cool-mist vaporizers were used to increase the humidity within the healing chambers (Figs. 2.1A, 2.4A), and although they represent a relatively insignificant source of material costs (Table 2.6), these devices may not be needed in larger healing chambers.

INDIRECT COSTS ASSOCIATED WITH GRAFTING

Indirect costs associated with grafting represent line items that were increased (per plant) in the grafted production sequence as compared to the nongrafted plants. Indirect costs such as additional soil, trays, labor, and heating were 46% and 51%
higher during the production of the grafted plants in NC and PA, respectively (Tables 2.4-5). These costs were particularly important in the PA study, and the combined indirect costs (misc. materials, heating, transplant labor) accounted for 10% and 30% of the added costs of grafting in NC and PA, respectively (Fig. 2.3). The majority of indirect costs of grafting were manifested as waste during the grafting procedure (Fig. 2.4C). These expenses could be reduced through innovative production techniques.

Micrografting has been recently introduced into the arena of tomato grafting and this technique uses micro-propagated scion grafted onto 3 week-old rootstock seedlings (Grigoriadis et al., 2005). For propagators unable to carryout this type of advanced procedure, similar principles of reducing indirect costs could be utilized.

LABOR COSTS OF GRAFTED TRANSPLANT PRODUCTION

Labor plays an important role in grafted and nongrafted transplant production budgets (Table 2.6) and contributes to the added cost of grafting directly and indirectly. In these case studies, manual grafting labor costs were $0.07 to $0.18 per plant (Tables 2.4-5). Using the same formula as in the PA sequence \(\frac{\$16.39/h}{\text{grafting rate} \times 90\% \text{ success}}\) combined with current manual grafting rates (Kubota et al., 2008), the cost of the manual grafting procedure could be as low as $0.03 to $0.06 per plant.

Although the per plant cost of manual grafting in our studies was relatively high, it did not increase the proportional labor costs of production in comparison to nongrafted plants. Interestingly, the proportional labor costs were lower in the grafted
sequence in NC and similar in the PA study (Table 2.6). This effect is due to the additional material and seed costs associated with grafting in both of these studies (Tables 2.4-5). These results suggest that at current seed prices and under similar production methods, labor prices would have little or no effect on the feasibility of tomato grafting in U.S. transplant production systems compared to other factors such as rootstock seed and other indirect costs.

Although the price of labor in the US may not have a strong proportional effect on the variable costs incurred for domestic production, the sheer volume of manual labor required for tomato grafting could be difficult to obtain. In the PA study, grafting wages were increased to $14/h to coincide with skilled labor wages. This may be one way to make labor more available for grafting. It could also be useful for propagators to market their clientele in a way that enables numerous moderately-sized batches of grafted plants rather than attempting to produce one or several large batches in a given season.

COSTS OF ORGANIC TRANSPLANT PRODUCTION

At the NC location, the grower produced certified organic transplants for use on the farm while the grower in PA did not. Interestingly, the introduction of organic certification into the NC production sequence made a negligible effect on the overall cost of transplant production. Management of a large-scale commercial organic transplant production facility may require added fixed and variable expenses that were
not seen at Black River Organic Farm. In this example, the greenhouse was previously certified, and many of the variable expenses related to propagation were unaffected by organic certification (e.g. labor, heating, plastic materials). Exceptions to this include potting media, fertilizer, and certified or untreated seed. In this case, untreated seed was used and the cost of organic fertilizers was similar to those used in the PA study. Assuming the price of the “conventional” potting media used in the PA study (Table 2.2) in comparison to the price of the OMRI-listed potting media used for the NC study (Table 2.1), the additional soil costs associated with the organic potting media in the NC sequence added ~$0.01 to $0.02 per transplant.

BENEFITS OF GRAFTED TRANSPLANT PRODUCTION IN THE U.S.

At the NC location, plants were raised for on-farm tomato fruit production (Fig. 2.4A-B) and the benefit of grafted propagation translated to advantages associated with the utilization of the grafted plants. This grower had experienced repeated crop failures due to soilborne disease problems. The fruit production and economic benefits associated with the utilization of the grafted plants from this study have been provided in a recent report (O’Connell et al., 2009b). The deployment of resistant rootstocks to manage these diseases resulted in substantially higher per acre profit and ultimately allowed this grower to retain organic tomato fruit sales for retail and wholesale markets (O’Connell et al., 2009b).
The case study from PA provides a better perspective of how grafting may benefit specialized plant propagators that are interested in the production and sales of grafted plants. Grafting could provide a new niche for propagators to explore through retail and commercial sales, but the market will ultimately dictate the amount of profit recovered by their sale.

At the PA location, the grafted plants provided a more profitable use of greenhouse space to the transplant grower than the nongrafts. In this production sequence, heating costs were directly related to the amount of space needed for the crop (Table 2.2). For every $1.00 the grower invested in greenhouse heating, nongrafted plants and grafted plants yielded $2.88 and $4.54 in wholesale mark-up, respectively (Table 2.5). Therefore, the grafted plants provided a more efficient use of greenhouse space. Although this trend would be similar regardless of the specific mark-up value (%), it is reliant upon the assumption that mark-up of grafted and nongrafted plants is equal. Currently, there is little market supply of grafted transplants in the U.S., but this new specialty market may help U.S. propagators retain profitability through wholesale and retail sales.

ECONOMIC MODELS AS A PRACTICAL TOOL

Models similar to the ones presented in Figures 2.2A-B can be used to compare the cost of grafted plants at various production stages, and therefore serve as a tool to predict the cost of grafted transplants for a variety of fruit production systems. In the
southeastern U.S., smaller transplants (3 to 5 true leaf stage) are utilized for open-field production as compared to the larger plants (6 to 10 true leaf stage) utilized for tunnel production in the PA study. By omitting the 18-cell growth stage in the PA sequence, equivalently-sized grafted plants (3 to 5 leaf stage) at the PA location were $1.02 while those at the NC location were $0.59 per plant (Figs. 2.2A-B). The range of values ($0.59 to 1.02) seen in these case studies represent a fair estimate of transplant costs a grower might experience in the U.S. for production of a similar-sized, grafted transplant (~6 weeks old), prior to mark-up.

This approach to illustrating costs of seedling production can also be used to identify areas along the transplant production sequence that could be considered for reducing the cost of grafting. For example, the discrete and continuous costs of grafted transplant production were twice that of the nongrafted plants prior to grafting and this can be visually observed in the PA sequence (Fig. 2.2B). Similar analyses of other production sequences may be used to determine areas where the additional costs of grafting could be addressed. Furthermore, such models could be adopted to help growers conceptualize profitable transplant production systems.
CONCLUSIONS

The case studies presented here are the first report of the variable costs of grafted transplant production in the U.S. and they represent two contrasting models of transplant production. Our results show that prior to mark-up, the utilization of grafting for tomato production could add $0.46 to $0.74 per plant, and including a 50% mark-up, this additional cost could be $1.12 per plant. Our findings are generally higher than those found in Morocco (Besri, 2003), but are consistent with the estimated additional costs provided in a recent review (Kubota et al., 2008).

U.S. growers and university personnel are concerned about the high cost of labor and its relevance on grafting. However, it is important to note that many of the commercial propagation facilities utilizing manual grafting worldwide are located in countries where labor prices are similar or only slightly less than in the U.S. The average wages of Canadian agricultural field workers in 2003-2008 ranged from $10.85-$13.04 (International Labor Organization, 2010) and other industrialized countries like Japan, Spain, and Italy currently produce grafted transplants without grafting robots (Besri, 2003, 2007; Kubota et al., 2008; Lee, 2003; Minuto and Causarano, 2008). In these case studies, we found that grafting labor costs made up a relatively small portion of the added cost of grafting, and labor costs were proportionally lower or similar among grafted plants as compared to nongrafted ones. These results do not suggest that the per plant cost of manual tomato grafting labor in the U.S. is negligible. However, they show strong evidence that labor prices in the U.S.
may not be as important as previous speculations have suggested and that efforts to reduce the immediate cost of grafted plants may be better-suited elsewhere. In both of these case studies, seed costs represented the highest additional cost of grafting and other factors such as grafting supplies and indirect costs played important roles in the additional cost of grating.

An important consideration for growers wishing to implement grafting for tomato fruit production is whether plants will be purchased or grown on the farm. In the PA case study, the per plant mark-up cost that a tomato fruit grower would pay was ~150% higher using grafted plants than nongrafted ones, and this factor could deter growers from buying grafted plants. Similarly, the initial expense of grafted transplants may reduce the likelihood of adoption in some growing systems and our results showed that the total per plant increase in transplant cost ranged from 64%-354%. Clearly, further work that ascertains any economic benefits of grafting in tomato fruit production will be of value to growers in the U.S.

The objectives of this study were not to design an optimum propagation system or to criticize current ones. Conversely, the goal was to explore two examples of successful grafted transplant propagation facilities and report their results. The specific cost a propagator may encounter is dependent not only on the level of care given, but also environmental conditions at the facility and current seed and labor costs. In a similar way, the benefit of grafting for commercial propagators will ultimately be driven by market demand for grafted plants. Worldwide, grafting has been utilized particularly
in greenhouses and high tunnels (Kubota et al., 2008; Lee, 1994) and preliminary reports suggest that tunnel growers in the U.S. would benefit from grafted plants (Groff, 2009a, 2009b; O’Connell et al. 2009a; Rivard et al. 2008b). The adoption of high tunnels in the U.S. (Carey et al., 2009) and the expansion of retail markets for grafted plants could be a valuable avenue for propagators looking to establish grafted transplant sales.
Table 2.1. Prices ($) of materials for the production of grafted and nongrafted tomatoes at Black River Organic Farm (Ivanhoe, North Carolina).

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Unit</th>
<th>Price(^{v}) ($/unit)</th>
<th>Lifespan(^{x}) (no. batches)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Seed costs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celebrity F1 untreated</td>
<td>scion / nongraft seed</td>
<td>1000 seeds</td>
<td>30.45</td>
<td></td>
<td>Harris Seeds (Rochester, NY)</td>
</tr>
<tr>
<td>RST-04-105-T(^{w})</td>
<td>rootstock seed</td>
<td>1000 seeds</td>
<td>175.00</td>
<td></td>
<td>Clifton Seed Company (Faison, NC)</td>
</tr>
<tr>
<td><strong>Direct grafting costs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0 mm(^{v}) silicone grafting clip</td>
<td>silicone tube grafting clip</td>
<td>200 clips</td>
<td>13.95</td>
<td></td>
<td>Johnny's Seeds (Winslow, ME)</td>
</tr>
<tr>
<td>Grafting knives, alcohol, etc</td>
<td>tools used during grafting</td>
<td>per operator</td>
<td>2.00</td>
<td>3</td>
<td>local / regional dept store</td>
</tr>
<tr>
<td>Rebar, fabric, plastic, blocks</td>
<td>chamber materials(^{a})</td>
<td>per chamber(^{a})</td>
<td>21.21</td>
<td>3</td>
<td>local / regional horticultural supply</td>
</tr>
<tr>
<td>Cool-mist vaporizer</td>
<td>1 per chamber(^{a})</td>
<td>per chamber(^{a})</td>
<td>36.00</td>
<td>3</td>
<td>local / regional dept store</td>
</tr>
<tr>
<td><strong>Other material costs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic Sunshine Mix</td>
<td>potting media(^{t})</td>
<td>per tray</td>
<td>0.60</td>
<td></td>
<td>Sun Gro Horticuture (Bellevue, WA)</td>
</tr>
<tr>
<td>512 cell plug tray</td>
<td>seedling tray</td>
<td>case (100)</td>
<td>40.00</td>
<td></td>
<td>local / regional horticultural supply</td>
</tr>
<tr>
<td>Re-useable plastic tray</td>
<td>50-cell tray</td>
<td>per tray</td>
<td>2.00</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Heating (propane)</td>
<td>greenhouse heating</td>
<td>per gal(^{b})</td>
<td>0.80</td>
<td></td>
<td>local propane supply</td>
</tr>
<tr>
<td>Heating (electric heater)</td>
<td>germination and chamber(^{a})</td>
<td>per hour</td>
<td>0.08</td>
<td></td>
<td>local electrical supply</td>
</tr>
</tbody>
</table>

\(^{v}\) Adapted from (O'Connell et al., 2009b).

\(^{v}\) Based on prices during budget development (Fall 2009). Prices in U.S. dollars.

\(^{x}\) Lifespan refers to no. batches of grafted transplants that were used to depreciate reusable items in the production sequence. Three batches were produced during spring 2008.

\(^{w}\) DP Seeds, Yuma, AZ.

\(^{v}\) 2.0 mm = 0.079 inch.

\(^{a}\) Once grafted, tomato transplants were placed in a healing chamber that holds 400 plants for 7 d.

\(^{t}\) Organic fertilizers (e.g. bone meal, feather meal, etc.) were incorporated into potting media.

\(^{s}\) $1.00/gal = $0.2642/L.
<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
<th>Unit</th>
<th>Price(^z) ($/unit)</th>
<th>Lifespan(^y) (no. batches)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed costs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BHN 589(^x)</td>
<td>scion / nongraft seed</td>
<td>1000 seeds</td>
<td>62.50</td>
<td></td>
<td>Siegers Seed Co. (Holland, MI)</td>
</tr>
<tr>
<td>Maxifort(^w)</td>
<td>rootstock seed</td>
<td>1000 seeds</td>
<td>194.46</td>
<td></td>
<td>Johnny’s Seeds (Winslow, ME)</td>
</tr>
<tr>
<td>Direct grafting costs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0 mm(^v) silicone grafting clip</td>
<td>silicone grafting clip</td>
<td>200 clips</td>
<td>8.40</td>
<td></td>
<td>Hydro-gardens (Colorado Springs, CO)</td>
</tr>
<tr>
<td>Grafting knives, alcohol, etc</td>
<td>grafting tools</td>
<td>per operator</td>
<td>2.00</td>
<td>3</td>
<td>local / regional dept store</td>
</tr>
<tr>
<td>Wire hoops, fabric, plastic</td>
<td>chamber(^n) materials</td>
<td>per chamber(^n)</td>
<td>29.50</td>
<td>3</td>
<td>local / regional horticultural supply</td>
</tr>
<tr>
<td>Cool-mist vaporizer</td>
<td>4 per chamber(^n)</td>
<td>per chamber(^n)</td>
<td>320.00</td>
<td>5</td>
<td>&quot;</td>
</tr>
<tr>
<td>Ebb-and-flood bench</td>
<td>chamber(^n) materials</td>
<td>sq. ft(^t)</td>
<td>3.50</td>
<td>10</td>
<td>&quot;</td>
</tr>
<tr>
<td>Other material costs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Custom plug costs</td>
<td>seedling germination</td>
<td>per seed</td>
<td>0.05</td>
<td></td>
<td>local custom propagator</td>
</tr>
<tr>
<td>Potting media</td>
<td>per 50-cell tray</td>
<td>per tray</td>
<td>0.28</td>
<td></td>
<td>local / regional horticultural supply</td>
</tr>
<tr>
<td>Potting media</td>
<td>per 18-cell tray</td>
<td>per tray</td>
<td>0.45</td>
<td></td>
<td>&quot;</td>
</tr>
<tr>
<td>50-cell trays</td>
<td>50-cell tray</td>
<td>case (100)</td>
<td>45.00</td>
<td></td>
<td>&quot;</td>
</tr>
<tr>
<td>1801 deep tray</td>
<td>18-cell tray</td>
<td>case (100)</td>
<td>66.00</td>
<td></td>
<td>&quot;</td>
</tr>
<tr>
<td>Web trays</td>
<td>18-cell tray only</td>
<td>case (100)</td>
<td>36.00</td>
<td></td>
<td>&quot;</td>
</tr>
<tr>
<td>Water-soluble fertilizer</td>
<td>applied through injector</td>
<td>per tray per week</td>
<td>0.03</td>
<td></td>
<td>&quot;</td>
</tr>
<tr>
<td>Heating</td>
<td>all greenhouse heat</td>
<td>per tray per day</td>
<td>0.07</td>
<td></td>
<td>&quot;</td>
</tr>
</tbody>
</table>

\(^z\) Based on prices during budget development (Fall 2009). Prices in U.S. dollars.
\(^y\) Lifespan refers to no. batches of grafted transplants that were used to depreciate reusable items in the production sequence. Three batches were produced during spring 2009.
\(^x\) BHN Seed, Immokalee, FL.
\(^w\) De Ruiter Seeds, Bergschenhoek, The Netherlands.
\(^v\) 2.0 mm = 0.079 inch.
\(^u\) Once grafted, tomato transplants were placed in a healing chamber that holds 3300 plants for 7 d.
\(^t\) $1.00/ft= $10.76/m
Table 2.3. List of variable costs during grafted and nongrafted tomato transplant production.

<table>
<thead>
<tr>
<th>Discrete&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Continuous&lt;sup&gt;x&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>plug costs (PA only)</td>
<td>daily watering</td>
</tr>
<tr>
<td>potting mix</td>
<td>heating costs</td>
</tr>
<tr>
<td>seedling trays</td>
<td>transplant care / scouting</td>
</tr>
<tr>
<td>transplanting labor</td>
<td>weekly fertilizer (PA only)</td>
</tr>
<tr>
<td>grafting facility</td>
<td>healing chamber costs</td>
</tr>
<tr>
<td>grafting equipment</td>
<td>humidifier costs</td>
</tr>
<tr>
<td>grafting clips</td>
<td>healing chamber care</td>
</tr>
<tr>
<td>grafting labor</td>
<td></td>
</tr>
</tbody>
</table>

<sup>z</sup> Variable costs were itemized in production sequences from two commercial farms in North Carolina and Pennsylvania (PA). Discrete and continuous expense categories were utilized for the development of Figs. 2A-B.

<sup>y</sup> Discrete variable costs include line items that add to production costs as individual items or events.

<sup>x</sup> Continuous variable costs include line items that accumulate over time during transplant production.
**Table 2.4. Variable costs of tomato transplants ($/1000 plants) at Black River Organic Farm (Ivanhoe, North Carolina).**

<table>
<thead>
<tr>
<th>Description</th>
<th>Nongrafted</th>
<th>Grafted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Materials$^z$</td>
<td>Labor$^z$</td>
</tr>
<tr>
<td>Seed costs$^w$</td>
<td>233.35</td>
<td></td>
</tr>
<tr>
<td>Rootstock (RST-04-105-T)$^v$</td>
<td>36.00</td>
<td>40.00</td>
</tr>
<tr>
<td>Scion (Celebrity F1)$^a$</td>
<td>10.56</td>
<td>19.84</td>
</tr>
<tr>
<td>Plastic trays</td>
<td>21.65</td>
<td>43.30</td>
</tr>
<tr>
<td>Heating</td>
<td>16.56</td>
<td>16.56</td>
</tr>
<tr>
<td>Transplanting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transplant care</td>
<td>77.23</td>
<td></td>
</tr>
<tr>
<td>Manual grafting$^c$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grafting clips</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>Misc. supplies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healing chamber$^r$</td>
<td>20.21</td>
<td>8.28</td>
</tr>
<tr>
<td>Chamber supplies</td>
<td>0.13</td>
<td>0.59</td>
</tr>
<tr>
<td>Total (materials and labor)</td>
<td>94.40</td>
<td>38.21</td>
</tr>
<tr>
<td>Cost per plant</td>
<td>132.61</td>
<td>587.59</td>
</tr>
</tbody>
</table>

$^z$ Adapted from (O’Connell et al., 2009b).

$^v$ Based on prices during budget development (Fall 2009). Prices in U.S. dollars.

$^a$ Based on average hourly agricultural wages (U.S. Department of Agriculture, 2009).

$^w$ Seed costs were calculated to reflect the total cost required for 20% over-sowing and 90% grafting success (where applicable).

$^v$ Inter-specific rootstock cultivar (DP Seeds, Yuma, AZ). Source: Clifton Seeds, Faison, NC.

$^a$ Determinate fresh-market variety. Source: Harris Seeds, Rochester, NY.

$^c$ Bone meal, feather meal and other organic amendments were incorporated into potting mix.

$^r$ Grafting rate was 200 plants/worker/h and grafting wage was $10.08/h. Success rate was 90%.

$^r$ Once grafted, tomato transplants were placed in a healing chamber that holds 400 plants for 7 d.
Table 2.5. Variable costs of tomato transplants ($/1000 plants) at Good Harvest Farms (Strasburg, Pennsylvania).

<table>
<thead>
<tr>
<th>Description</th>
<th>Nongrafted</th>
<th>Grafted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Materials$^a$</td>
<td>Labor$^a$</td>
</tr>
<tr>
<td>Seed costs$^x$</td>
<td>Rootstock (&quot;Maxifort&quot;)$^w$</td>
<td>72.92</td>
</tr>
<tr>
<td></td>
<td>Scion (&quot;BHN 589&quot;)$^v$</td>
<td>78.13</td>
</tr>
<tr>
<td>Transplant production</td>
<td>Custom plug costs$^z$</td>
<td>57.60</td>
</tr>
<tr>
<td></td>
<td>Potting mix</td>
<td>57.60</td>
</tr>
<tr>
<td></td>
<td>Plastic trays</td>
<td>30.65</td>
</tr>
<tr>
<td></td>
<td>Heating</td>
<td>65.78</td>
</tr>
<tr>
<td></td>
<td>Transplanting</td>
<td>88.41</td>
</tr>
<tr>
<td></td>
<td>Transplant care</td>
<td>73.69</td>
</tr>
<tr>
<td>Grafting</td>
<td>Manual grafting</td>
<td>5.68</td>
</tr>
<tr>
<td></td>
<td>Grafting clips</td>
<td>112.30</td>
</tr>
<tr>
<td></td>
<td>Misc. supplies</td>
<td>6.96</td>
</tr>
<tr>
<td>Healing chamber$^y$</td>
<td>Chamber supplies</td>
<td>13.33</td>
</tr>
<tr>
<td>Total</td>
<td>321.04</td>
<td>187.36</td>
</tr>
<tr>
<td>Total (materials and labor)</td>
<td>1525.20</td>
<td>1252.28</td>
</tr>
<tr>
<td>Cost per plant</td>
<td>0.51</td>
<td>1.25</td>
</tr>
<tr>
<td>Selling price (50% mark-up)</td>
<td>0.76</td>
<td>1.88</td>
</tr>
</tbody>
</table>

$^a$ Based on prices during budget development (Fall 2009). Prices in U.S. dollars.
$^b$ Based on average hourly agricultural wages (U.S. Department of Agriculture, 2009).
$^x$ Seed costs were calculated to reflect the total cost required for 20% over-sowing and 90%
     grafting success (where applicable).
$^w$ Inter-specific rootstock (De Ruiter Seeds, Bergschenhoek, The Netherlands). Source: Johnny's
     Selected Seeds, Winslow, ME.
$^v$ Determinate fresh-market variety (BHN Seed, Immokalee, FL). Source: Siegers Seed Co., Holland, MI
$^z$ Seedlings were germinated by a local custom plug propagator (York, PA).
$^t$ Grafting rate was 100 plants/worker/h and grafting wage was $14.00/h. Success rate was 90%.
$^y$ Once grafted, tomato transplants were placed in a healing chamber that holds 3300 plants for 7 d.
Table 2.6. Distribution of variable costs (%) for tomato transplant production at Black River Organic Farm (Ivanhoe, North Carolina) and Good Harvest Farms (Strasburg, Pennsylvania).

<table>
<thead>
<tr>
<th>Description</th>
<th>NC location</th>
<th>PA location</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nongrafted</td>
<td>Grafted</td>
</tr>
<tr>
<td>Seed*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scion</td>
<td>27.1</td>
<td>6.8</td>
</tr>
<tr>
<td>Roostock</td>
<td>39.7</td>
<td></td>
</tr>
<tr>
<td>Total seed costs</td>
<td>27.1</td>
<td>46.5</td>
</tr>
<tr>
<td>Labor^</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transplanting</td>
<td>16.3</td>
<td>7.4</td>
</tr>
<tr>
<td>Transplant care</td>
<td>12.5</td>
<td>2.8</td>
</tr>
<tr>
<td>Manual grafting^x</td>
<td>11.1</td>
<td>14.4</td>
</tr>
<tr>
<td>Chamber construction</td>
<td>1.4</td>
<td>0.3</td>
</tr>
<tr>
<td>Total labor costs</td>
<td>28.8</td>
<td>22.7</td>
</tr>
<tr>
<td>Materials^w</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plug costs^v</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heating costs</td>
<td>24.1</td>
<td>5.4</td>
</tr>
<tr>
<td>Potting mix</td>
<td>11.9</td>
<td>5.1</td>
</tr>
<tr>
<td>Plastic trays</td>
<td>8.0</td>
<td>3.4</td>
</tr>
<tr>
<td>Grafting clips</td>
<td></td>
<td>13.1</td>
</tr>
<tr>
<td>Grafting tools</td>
<td></td>
<td>0.3</td>
</tr>
<tr>
<td>Chamber materials</td>
<td></td>
<td>3.4</td>
</tr>
<tr>
<td>Total material costs</td>
<td>44.0</td>
<td>30.8</td>
</tr>
</tbody>
</table>

* Seed costs were calculated to reflect the total cost required for 20% over-sowing and 90% grafting success (where applicable).

^ Based on average hourly agricultural wages (U.S. Department of Agriculture, 2009).

^x Grafting speed was 200 and 100 plants/worker/hour at the NC and PA locations, respectively. Grafting wages were $10.08/h in NC and $14.00/h in PA.

^w Based on prices during budget development (Fall 2009). See Tables 2.1-2.2 for details.

^v Seedlings were germinated by a local custom plug propagator (York, PA).
Figure 2.1 – Healing chambers used for grafted tomato production at (A) Black River Organic Farm and (C) Good Harvest Farms. B) Seedling propagation at Black River Organic Farm. D) “Twin-leader” transplant two weeks prior to sale/planting.
Figure 2.2 - Economic timeline of a grafted and non-grafted tomato transplants at (A) Black River Organic Farm (Ivanhoe, NC) and (B) Good Harvest Farms (Strasburg, PA). Rectangles and triangles scaled to represent discrete (e.g. transplanting labor, materials, grafting labor, clips, etc) and continuous (e.g. overhead, daily watering labor, fertilizer, etc) expenses, respectively (See Table 2.3).
Figure 2.3 – Distribution of the added cost of grafting at the NC and PA locations. Additional per plant cost of grafting was $0.46 in NC and $0.74 in PA.
Figure 2.4 – Newly grafted plants (A) and organic tomato production area adjacent to propagation greenhouses (B) at Black River Organic Farm. C) Manual grafting at Good Harvest Farms. D) “Twin-leader” transplants in production at Cedar Meadow Farm (Photo courtesy: Steve Groff).
LITERATURE CITED


ACKNOWLEDGEMENTS

The authors would sincerely like to thank Stefan Hartmann (Black River Organic Farm), Chris Powell (Good Harvest Farms), Steve Groff (Cedar Meadow Farm), Dr. Michael Orzolek (Penn State Univ), and Penn State Extension for cooperation on this project. Special thanks to Noel Mooney, Kaitlin Dye, and Dana Groff. Funding provided by the Organic Farming Research Foundation, Northeast Region SARE FNE09-658, Southern Region SARE LS06-193 and GS07-060, and USDA-NIFA 2007-51106-03794.
CHAPTER THREE

The following report

Grafting for organic open-field and high tunnel production

of heirloom tomatoes

by

C.L. Rivard, S. O’Connell, C.D. Harlow, M.M. Peet, and F.J. Louws

was prepared for submission to

HortScience

The American Society for Horticultural Science

Alexandria, VA
ABSTRACT

Grafting with resistant rootstocks is a valuable disease management strategy, particularly for organic heirloom tomato (*Solanum lycopersicum*) growers, but little information is available on the ability of rootstocks to increase yields in US production systems that have non-infested soils. Additionally, high tunnels have gained popularity in the US and this report explores the utility of grafting for organic high tunnel and open-field tomato production using the heirloom cultivar ‘Cherokee Purple’ (CP). In both years, the main effect of rootstock was significant (*P*<0.01) and the grafted plants responded similarly in the open-field and high tunnel systems. Grafting CP with ‘Beaufort’ and ‘Maxifort’ rootstocks increased yield by 23% to 54% (*P*<0.05). Increases in fruit yield were the result of increased number and/or size and these factors accounted for 50% and 48% of the variability, respectively. Increases in fruit yield were seen early to mid-way through the harvest season (*P*<0.05), and grafting CP with ‘Beaufort’ and ‘Maxifort’ rootstocks increased fruit yield in both systems 100 days after planting (*P*<0.05). In the open-field system, CP plants grafted onto ‘Maxifort’ rootstocks and grown at reduced planting densities had similar or higher per hectare fruit yield compared to nongrafted plants grown at the standard planting density (*P*<0.05). ‘Big Power’ and ‘RST-04-105-T’ rootstocks generated intermediate yields compared to ‘Beaufort’ and ‘Maxifort’ rootstocks and the nongrafted controls in the open-field.

Grafting will be an important component for growers that wish to reduce risk of disease.
outbreaks and this report suggests that yield increases in high tunnel and open-field systems can be seen when disease pressure is low.
INTRODUCTION

Organic heirloom tomatoes can be a profitable crop for growers who cater to local wholesale and retail markets (Grassbaugh et al., 1999; Lin, Smith, and Huang, 2008), but production can be difficult. Heirloom cultivars are open-pollinated and many were released more than 50 years ago (DeMuth, 1998), prior to the discovery of most major resistance genes that are typically utilized in hybrid cultivars. Heirloom varieties typically have little resistance to known pests and pathogens and many have poor production characteristics. However, this market is consumer-driven (DeMuth, 1998) and prices of heirloom varieties in specialty markets can be very high (Jordan, 2007). A recent survey found that approximately half of the 50 plus articles featuring heirloom tomatoes were in restaurant reviews (Jordan, 2007). Although this type of media attention is an excellent way to expand this niche for growers, it results in consumers that seek out specific cultivars from the market and some of these may have poor production qualities, particularly in an organic production setting where host resistance is crucial to an integrated pest management program (Chellemi, 2002).

Examples of heirloom cultivars that are typical of organic production in NC are ‘Cherokee Purple’, ‘German Johnson’, ‘Mr. Stripey’, ‘Brandywine’, and ‘Kellogg’s Breakfast’ (Rivard and Louws 2008; Rivard and Louws, 2006).

In the southeastern US, organic heirloom production is particularly difficult due to severe disease pressure as well as the presence of a challenging growing environment. A recent report indicates that high tunnel (hoop house) production
reduces risk of foliar disease epidemics in organic heirloom tomato production (O’Connell et al., 201X). High tunnels have expanded rapidly throughout the US (Carey et al., 2009; Lamont et al., 2003) and worldwide (Lamont, 2009; Wittwer and Castilla, 1995). High tunnels (hoop houses) can be constructed from standard greenhouse frames and are generally less expensive than traditional greenhouse production (Wells and Loy, 1993). Plants are grown in the soil and passive thermal protection is utilized (Carey et al., 2009).

Although high tunnel production can be favorable for organic heirloom tomato production in the southeastern US, the cost of these structures may force growers to reduce crop rotation intervals ultimately resulting in increased soilborne pathogen inoculum over time (Beckerman, 2004; Kaskavalci et al., 2009). Grafting on resistant rootstock is an emerging technology in US tomato production and has been proposed as a way to reduce losses by soilborne diseases (King et al., 2008; Kubota et al., 2008), particularly for organic and heirloom growers (Kaskavalci et al., 2009; Rivard and Louws 2008a).

Recently, ‘Big Power’, ‘Beaufort’, and ‘Maxifort’ rootstocks were shown to significantly reduce the incidence of southern blight (caused by Sclerotium rolfsii) when grafted with ‘Cherokee Purple’ and ‘German Johnson’ heirloom scion (Rivard et al., 2010). ‘Big Power’ also showed excellent resistance to southern root-knot nematode (Meloidogyne incognita) and ‘Beaufort’ and ‘Maxifort’ had intermediate resistance (Rivard et al., 2010). Many rootstocks carry major resistance genes against F.
oxysporum f.sp. lycopersici, and ‘Maxifort’ was resistant to fusarium wilt in a study with ‘German Johnson’ scion (Rivard and Louws, 2008). Southern bacterial wilt (caused by Ralstonia solanacearum) is a particularly severe problem in the southeastern US, and grafting with ‘RST-04-105-T’ rootstock and others has shown promise for growers with infested soils (Freeman, Rideout, and Wimer, 2009; Rivard et al., 2008b).

In addition to providing resistance to soilborne plant pathogens, grafting may also confer other advantages in organic heirloom production, including tolerance to abiotic stress and increased plant vigor (Rivero, Ruiz, and Romero, 2003a). Worldwide, various rootstocks have been used to manage stressors such as soil salinity (Estan et al., 2005; Fernandez-Garcia et al., 2004), thermal stress (Abdelmageed and Gruda, 2009; Rivero, Ruiz, and Romero, 2003b; Venema et al., 2008) and flooding (Black et al., 2003).

Not only could tomato rootstocks provide tolerance to abiotic stress for heirloom production, but they may also affect other metabolic functions in the scion resulting in increased yield and crop productivity. Stomatal conductance (Fernandez-Garcia et al., 2002; He et al., 2009), photosynthetic activity (Abdelmageed and Gruda, 2009; He et al., 2009; Matsuzoe et al., 1993), nutrient uptake (Leonardi and Giuffrida, 2006; Rivero, Ruiz, and Romero, 2004; Ruiz and Romero, 1999), and general plant growth (Abdelmageed and Gruda, 2009; He et al., 2009; Loos, Caliman, and da Silva, 2009; Matsuzoe et al., 1993) are impacted by the implementation of vigorous rootstocks.
Changes in overall plant growth and vigor among grafted crops may lead to increased fruit yield even where no soilborne pathogen pressure or identified abiotic stress is evident (Loos, Caliman, and da Silva, 2009; Poganyi et al., 2005; Upstone, 1968; Yetisir and Sari, 2003). For example, grafting is most common in North America in highly-controlled hydroponic greenhouse operations (Kubota et al., 2008). It has been suggested that grafting with vigorous rootstock extends the length of the harvest period (Lee, 1994). In a recent trial with ‘German Johnson’, the cumulative yield benefit of using ‘Maxifort’ rootstock in the absence of soilborne disease pressure was not significant until the end of the season, and at the other two locations tested, fruit yield was not increased (Rivard and Louws, 2008).

Vegetable grafting is practiced extensively for protected cultivation worldwide, including high tunnels and greenhouses (Kubota et al., 2008; Lee, 1994, 2003; Oda, 1999). The extended growing season and potentially stressful conditions within the high tunnel system may be more conducive to the use of grafted plants. However, there are few reports that examine the benefit of grafting in the high tunnel compared to the open-field system and none have made this comparison with heirloom cultivars. In Greece, ‘Heman’ and ‘Primavera’ rootstocks plants performed similarly in the high tunnel and open-field, but data were collected for only one growing season and there was no effect of grafting in either system (Khah et al., 2006).

In some cases, the increased vigor seen in grafted plants has led to the adoption of decreased planting densities as a way to reduce the economic constraints of using
grafted plants. Greenhouse operations in the US have adopted a “twin-leader” production system (Kubota et al., 2008) and high tunnel operations in Morocco have adopted similar methods (Besri, 2003). These principles of reducing the costs of grafting while maintaining the benefits may be appropriate for heirloom growers, particularly in cases where yield increases are not consistent (Rivard and Louws, 2008a).

Although grafting could be advantageous for heirloom tomato growers in the US who face naturally-infested soils, little work has been done to determine if increased vigor provided by inter-specific rootstocks will result in higher fruit yield for heirloom production in non-infested soils. Furthermore, the influence of the production system (open-field vs high tunnel) on the utility of grafting is unclear.

In order to gain a better understanding of the importance of grafting and high tunnels for organic heirloom tomato production in the southeastern US, an inter-disciplinary study was carried out at the Center for Environmental Farming Systems (Goldsboro, NC) from Mar 2007 to Mar 2009. A comprehensive account of the impact of high tunnel production on foliar disease, environmental conditions, cover crop biomass production, and nongrafted crop yield can be found in O’Connell et al. (201X).

This report compares the specific role of grafting in high tunnel and open-field production systems and evaluates various planting densities of grafted plants in the open-field for organic heirloom tomato production in the southeastern US. Therefore, the objectives were 1) to determine if grafting with vigorous rootstocks affects fruit
yield in the absence of soilborne pathogens, 2) to compare the effect of grafting in the high tunnel versus the open-field system, and 3) to observe the effect of planting density on grafted plants grown in the open-field.
MATERIALS AND METHODS

_Grafting and transplant production._ All grafted and nongrafted transplants were produced at the Southeastern Plant Environment Laboratory located at North Carolina State University (Raleigh, NC; http://www.ncsu.edu/phytotron/). ‘Cherokee Purple’ (Johnny’s Selected Seeds; Winslow, ME USA) was used as the scion cultivar for rootstock treatments and for nongrafted controls. This open-pollinated cultivar is used regionally for fresh-market production, and has no known resistance to any economically-important plant pathogens. Commercially-available rootstock cultivars, 'Big Power' (Rijk Zwaan Seeds; De Lier, The Netherlands), 'Beaufort' (De Ruiter Seeds; Bergschenhoek, The Netherlands), 'Maxifort' (De Ruiter Seeds; Bergschenhoek, The Netherlands), and 'RST-04-105-T' (DP Seeds; Yuma, AZ USA) were used as rootstock treatments in the field plots and 'Beaufort' and 'Maxifort' were utilized in the tunnel treatments. ‘Big Power’, ‘Beaufort’, and ‘Maxifort’ are _S. lycopersicum_ L. x _S. habrochaites_ (S. Knapp and D.M. Spooner), and ‘RST-04-105-T’ is _S. lycopersicum_ L. x unknown _Solanum_ spp.

The Japanese tube grafting technique was carried out for all grafted plants (Lee, 2003; Oda, 1999; Rivard and Louws, 2006). Rootstock and scion seedling stems were severed and held together using a silicon clip. Once grafted, the plants were immediately moved into a "healing chamber" where humidity and light conditions were manipulated to promote graft union formation (Rivard and Louws, 2006). Once grafting and subsequent healing had occurred, grafted and nongrafted plants were transplanted
to 10 cm pots and allowed to grow for 14 days in the greenhouse before being planted into the experimental plots.

**Experimental Design.** Research trials were carried out in 2007 and 2008 at the Center for Environmental Farming Systems (long. 35° 24' 0.5" N, lat. 78° 01' 52.6" W). Two (9.1 m x 29.3 m) high tunnels were constructed using a snow-arch design greenhouse frame (Atlas Greenhouses Inc.; Alapaha, GA USA), and equivalent field plots were established 15 m adjacent to the high tunnels. The soil was a Wickham sandy loam (pH = 6.2) and the field had no history of tomato production or pathogens problematic on tomato. Although the land used for this study had not been organically certified, all aspects of open-field and high tunnel construction as well as production methods were performed under the guidelines of the National Organic Program (NOP) organic certification standards.

The data presented here were collected from three complementary experiments that were nested within a “systems” comparison of high tunnel vs open-field organic heirloom tomato production. In order to address objectives one and two, a factorial study was nested within the open-field and high tunnel systems and replicated four times. The system treatments (tunnel vs open-field) served as main plots and the three rootstock treatments (nongrafted, ‘Beaufort’, ‘Maxifort’) were randomized within the main plots.
Two additional experiments were nested within the open-field system to strengthen evidence pertaining to objective one and address objective three. In the open-field system, nongrafted ‘Cherokee Purple’ (CP) was compared to plants with ‘CP’ scion grafted onto ‘Big Power’ and ‘RST-04-105-T' rootstocks in addition to ‘Beaufort’ and ‘Maxifort’. Nongrafted CP and plants grafted with ‘Maxifort’ rootstocks were also grown in the open-field system at various in-row spacings to reflect standard, two-thirds-, and half-rate planting densities.

In all treatments, the experimental units consisted of a single 3.7 m row, and six plants were set at 61 cm in-row spacing for the treatments that received the standard planting density (11,960 plants/ha). In all trials, rows were spaced 1.4 m apart. The standard in-row spacing was utilized for all treatments and in both systems. One exception to this was the nongrafted CP and CP grafted onto ‘Maxifort’ that were trialed exclusively in the open-field at reduced planting densities (see below for details). Each treatment was replicated four times and crop management and data collection was carried out according to the replicated blocks.

**Implications of the systems comparison.** The goal of a “systems” comparison is to identify the net result of many elements when comparing two distinct systems (Ikerd, 1993). Due to the presence of systems- and component-level hypotheses in this study, many factors were imposed equivalently across the systems, including planting density, fertility, and general cultural methods. However, planting dates were scheduled to
correspond with optimum planting dates for regional tomato production (Ivors, 2010). Similarly, the plant training system employed in the open-field was distinct from that of the high tunnels and each training system was selected as the optimum management practice for that system. Another factor that varied slightly across the systems was irrigation. If daily rainfall accumulated to significant levels (>5 mm), then irrigation was reduced in the tunnels based on cloud cover and eliminated in the field. In both cases, irrigation was supplied to provide optimum soil moisture conditions according to the needs of the system. A detailed comparison of nongrafted plants and a comprehensive description of the growing environment (e.g. air and soil temperature, soil moisture, relative humidity) as well as foliar disease pressure in the high tunnel and open-field systems was presented by O’Connell et al. (201X).

**Similarities across the systems.** In both years, pre-plant activities, initial pruning and plant training, and scheduled fertigation events were performed with regard to the planting date imposed in each system. Nitrogen was supplied to the tomato crop at rates of 112 kg/ha in both years and pre-plant nitrogen applications were 99 and 93 kg/ha in 2007 and 2008, respectively. In 2007, 22 t/ha of compost (McGill Environmental Systems; Harrells, NC USA) was applied and incorporated to all plots 14 days prior to planting. Pre-plant application of 102 kg/ha of feathermeal (12-1-0, Nutrimax Inc.; Greensboro, NC USA) was applied to all plots in 2007.
Supplemental potassium and calcium additions were made based on leaf tissue analysis and soil test recommendations by the North Carolina Department of Agriculture and Consumer Services (http://www.agr.state.nc.us/agronomi/; Raleigh, NC). In 2008, pre-plant potassium applications were made in both systems by incorporating 145 kg/ha of K₂O (Crop Production Services; Collinsville, IL USA). Calcitic lime was applied at a rate of 2.3 t/ha to both systems on 6 Oct 2006 and 26 Sep 2007. In 2007, supplemental calcium was applied as a soluble foliar spray (Grow More Inc.; Gardens, CA USA) at a rate of 7.8 ml/l on 4 and 26 June.

Cover crops were sown equally and on the same day across the systems. Prior to the 2007 growing season, a winter rye (Secale cereale) cover crop was planted at a rate of 50 kg/ha on 11 Nov 2006. Prior to the 2008 growing season, winter rye and hairy vetch (Vicia villosa) were sown in mixture at rates of 34 and 50 kg/ha, respectively, and this crop was planted in both systems on 26 Sep 2007. Cover crop irrigation was provided by overhead sprinklers. Water was provided equally across the systems as needed during the initial four weeks of crop establishment and bi-weekly thereafter. In the field system, bi-weekly irrigation events were omitted when substantial rainfall events (>10 mm) within the preceding week had occurred.

Cover crops were incorporated 28 days prior to planting using a tractor-mounted rotary tiller. Tillage was repeated 14 days prior to planting, and soil amendments including compost, feathermeal and potassium were incorporated. Weed control was accomplished in the tomato crop was accomplished by polyethylene fabric
and hand-weeding as needed. Water and soluble nutrients were provided through drip irrigation. Irrigation events ranged from 0.5 to 2 hours daily, depending on environmental conditions and the growth stage of the crop. All scheduled fertigation was supplied equally to both systems and according to the planting dates.

**High tunnel production and management.** The high tunnel plots were planted on 20 Mar 2007 and 18 Mar 2008. Sidewall curtains were closed when nighttime temperatures were predicted to fall below 13 degrees C, and the sidewall curtains and endwall shutters were opened as necessary to provide adequate ventilation.

After planting in the high tunnels, the apical meristem was removed from each plant to induce the formation of two main stems that were trained vertically using a modified European “string-trellis” management system (Fuller, 1973). All suckers were removed weekly and the stems were trained to a vertical string with polyethylene plant clips (Hydro-gardens Inc., Colorado Springs, CO USA). Lower leaves were removed up to the leaf below the first fruit cluster (Fuller, 1973). In 2007, supplemental potassium (0-0-52; SQM North America Crop.; Atlanta, GA USA) was applied on 15 and 28 June at rates of 91.5 kg/ha and 70.4 kg/ha, respectively. In 2008, 91.5 kg/ha of potassium was provided on 8 July. Fertigation was performed on 4 and 18 May; and 1, 14, and 28 June in 2007 and 27 May and 10 June in 2008.
**Open-field production and management.** The field plots were planted on 19 Apr 2007 and 17 Apr 2008. Typical of regional open-field production, the plants were not pinched and the stake-and-weave training systems was employed (Ivors, 2010). Metal posts (2.1 m) were placed between every other plant and polyethylene string was wrapped around the posts and used to hold the plants upright. In 2007, supplemental potassium (0-0-52; SQM North America Crop.; Atlanta, GA USA) was applied on 15 and 28 June at rates of 91.5 kg/ha and 70.4 kg/ha, respectively. In 2008, 91.5 kg/ha of potassium was provided on 27 July. Fertigation was performed on 1, 14, and 28 June; and 12 and 26 July in 2007, and 24 June and 8 July in 2008.

**Experiments exclusive to the open-field.** In addition to the factorial “systems” experiment, two complimentary protocols were carried out in the open-field in order to address objectives one and three. In the first trial, two additional rootstocks were tested (‘Big Power’ and ‘RST-04-105-T’) for yield effects. A plant spacing trial was also conducted in which nongrafted CP and CP grafted with ‘Maxifort’ rootstock were grown at increased in-row spacings. Standard in-row plant spacing in the trials was 61 cm (11,960 plants/ha). Nongrafted CP and CP grafted onto ‘Maxifort’ rootstocks were also grown at 92 cm and 123 cm (2008 only) to coincide with two-thirds and half-rate planting densities, respectively. All plants utilized for the open-field trials were provided with 168 and 112 kg/ha of nitrogen in 2007 and 2008, respectively. It should be noted that in 2007, the fertility was higher in these plots than in the ones utilized for
the “systems” experiment. All pre-plant application methods and timing of fertigation were similar to those previously listed in the description of the open-field, but fertigation rates were increased equivalently to provide additional nitrogen to the crop in 2007.

**Data collection and statistical analysis.** Plots were maintained and evaluated by replication throughout the course of the season and weekly foliar and disease ratings were made to assess plant damage by pests and pathogens (See O’Connell et al., 201X). Fruit was harvested and graded twice weekly from 1 Jun to 13 Aug in 2007 and 30 May to 11 Aug in 2008. Total and marketable fruit number and weight were recorded for each harvesting event. In both years, the termination of the trials occurred on the same day, and this was 116 and 146 days after planting in the open-field and high tunnel systems, respectively. Upon termination, all fruit larger than 5 cm were harvested and recorded.

Yearly data from the three experiments were combined and the MIXED procedure was performed (SAS 9.1; SAS Institute, Cary, NC USA). An estimate of the means was generated using the LSMEANS statement and the means were compared using Tukey’s least significant difference test ($\alpha = 0.05$). Table 3.1 contains a detailed explanation of the experiments utilized and their corresponding main- and sub-plot treatments.
The high tunnel was planted 30 days prior to the open-field. Therefore, cumulative yield data from both years were normalized by planting date and analyzed using total fruit yield that had been collected 100 days after planting in both systems. However, in order to gain a “systems” perspective, final cumulative fruit yield data from the entire season (116 and 146 days in the open-field and high tunnel, respectively) was analyzed in a similar fashion to the data collected 100 days after planting.

In order to determine the effects of grafting on different periods during the harvest season, a repeated measures analysis was carried out using the MIXED procedure as well. Fruit was harvested for 9 weeks in the high tunnel and 13 weeks in the open-field. Therefore, the bi-weekly harvest data was collected into five arbitrary harvest intervals based on the growing system and analyzed using the REPEATED statement. The last harvest interval included data from the terminal harvest only. The first four intervals represent equivalent periods within each system. In the high tunnel system each interval period was three weeks and in the open-field the interval period was two weeks. In order to determine if mean separation occurred during a given harvest period, a SLICE effects statement was used to calculate a $P$-value for each harvest interval.

An analysis of fruit yield was conducted from all treatments within the systems factorial study to determine the impact of fruit size and fruit number on yield. Fruit yield (t/ha) was correlated with total fruit number and mean fruit size using the CORR procedure in SAS. Linear regression was then carried out using the REG procedure, and
a stepwise statement was utilized to determine an $R^2$ value to describe the effects of fruit size and number on total fruit yield.
RESULTS

System*year and system*rootstock*year interaction terms were significant for fruit weight. Therefore, data collected from each year of the study were analyzed independently and the resulting P-values for main and sub-plot treatments are shown in Table 3.1. An exception to this trend was in the case of the rootstock treatments grown exclusively in the open-field where rootstock*year interactions were not significant (Table 3.1). The main effect of rootstock on the incidence (%) of marketable fruit (by weight) was not significant in either year (Table 3.1). For the purpose of this report, total fruit yield data is presented here.

Comparison of grafting effects across systems. In order to control for the longer growing season that occurs in the high tunnel system, cumulative yield data that had been collected 100 days after planting were analyzed across the two systems. The main effect of grafting was significant in both years (P<0.05; Table 3.1) and grafting had a significant effect on mean fruit size and number in both systems (P<0.05; Table 3.1). There were no significant interactions in either year for any of the parameters 100 DAP (Table 3.1) indicating that grafting had a similar effect in the open-field and high tunnel systems when the extended season was controlled for during data analysis.

The main effects of rootstock 100 DAP show that yield benefits as a result of grafting CP with 'Beaufort' and 'Maxifort' rootstocks ranged from 21% to 42% (P<0.05; Table 3.2) when averaged across the two systems. In 2007, 'Maxifort' had the highest
yields and performed particularly well in the high tunnels (Fig. 3.1C), but was not statistically different than 'Beaufort' (Table 3.2). 'Maxifort' increased total yield in 2007 compared to the nongrafted plants whereas 'Beaufort' increased fruit yield in both years ($P<0.05$; Table 3.2) 100 days after planting.

In order to address the effect of grafting at the systems level, the tunnel crop was allowed a longer growing season compared to the open-field. Grafting had a similar effect across the systems, and the average yield increases when CP plants were grafted onto 'Beaufort' and 'Maxifort' rootstocks ranged from 30%-48% ($P<0.05$; Table 3.2). The system*rootstock interaction terms for the full data sets were not significant and the main effects of grafting were significant in both years ($P<0.01$; Table 3.1).

In 2007, CP grafted with 'Maxifort' rootstock had the highest total fruit yield in both systems and performed particularly well in the high tunnels. Yield increases as a result of grafting with 'Maxifort' were 43% and 54% in the open-field and high tunnel, respectively ($P<0.05$; Fig. 3.3C). Among the two systems and both years, these were the highest proportional increases in yield seen in this study (Fig. 3.3C, 3.4C). Even with a growing season that was 30 days longer, the nongrafted plants in the high tunnels had lower yields than the ones in the open-field in 2007 ($P=NS$; Fig 3.3C) while in 2008 the nongrafted plants in the high tunnel had higher fruit yield than similar plants grown in the open-field ($P<0.05$; Fig. 3.4C). In 2008, 'Beaufort' and 'Maxifort' rootstocks increased fruit yield by 37% and 31% respectively, ($P<0.05$; Table 3.2). Although the
two rootstocks had statistically similar fruit yield, ‘Maxifort’ had the highest yield in 2007 and ‘Beaufort’ had highest yield in 2008 (Table 3.2).

Increased total fruit yield could be the result of increased fruit size and/or number. Fruit number was impacted differentially among the systems in 2007 (P<0.05; Table 3.1), and CP plants grafted onto ‘Maxifort’ rootstock had increased fruit numbers in the high tunnels (P<0.05; Fig. 3.3B). Fruit number was not impacted by rootstock in 2008 (Table 3.1). Interestingly, fruit size was not impacted in 2007 by grafting within any of the analyses whereas significant effects were prevalent in 2008 (P<0.05; Table 3.2).

In an effort to further describe the relationship between fruit yield increases seen among ‘Beaufort’ and ‘Maxifort’ rootstock and fruit number and/or size, a correlation analysis was conducted across both systems and both years. Total fruit weight was highly correlated to both fruit size and number (P<0.001). Fruit number and mean fruit size accounted for 50% and 48% of the variability within the yield data, respectively, and these results indicate that increases in fruit yield are explained relatively equally by increased fruit size and number.

Bi-weekly fruit yield data were organized into five arbitrary harvest intervals and analyzed for yield effects during each period of the growing season (Fig. 3.5). ‘Beaufort’ and ‘Maxifort’ rootstocks had significant effects on crop yield throughout the course of the season. A significant rootstock*harvest interaction occurred in the 2008 open-field system (P<0.05; Table 3.1). However, in the 2007 open-field system and both
years of the high tunnels the rootstocks had similar effects across all harvest periods of
the season (Table 3.1).

In the 2008 open-field system and in both years of the high tunnel trials, the
second harvest period was the only one that had significant effects of grafting within a
given harvest period \( (P<0.05; \text{Fig. 3.5B-D}) \). In the open-field system, the benefit of
grafting occurred throughout the harvest periods in 2007, but in 2008 the benefit of
grafting was most visible in the second and fifth harvest periods. In the high tunnel
system, the results were more consistent and CP plants grafted with ‘Beaufort’ and
‘Maxifort’ rootstocks increased fruit yield during the second harvest period in both
years \( (P<0.05; \text{Fig. 3.5C-D}) \).

**Grafting for the open-field.** Nongrafted ‘CP’ and ‘CP’ scion grafted onto ‘Maxifort’
rootstocks were grown at various in-row spacings in the open-field to determine how
grafted heirloom tomatoes would respond to reduced planting densities. The main
effect of rootstock on crop yield was significant \( (P<0.05) \) in both years and the
spacing*rootstock interaction was not (Table 3.1). ‘Maxifort’ increased fruit yield by
17% to 19% in the open-field when averaged across the various plant spacings \( (P<0.05; \text{Table 3.2}) \). In 2008, increases in fruit yield were most likely the result of increased fruit
size, \( (P<0.05; \text{Table 3.2}) \), and the ‘Maxifort’ plants produced similar sized fruit at all
planting densities (Table 3.3). Similarly, plant spacing did not have an effect on per
hectare fruit yield in either year (Table 3.1). Furthermore, in 2007, CP grafted with
‘Maxifort’ rootstock at 7,970 plants/ha had higher fruit yield than nongrafted CP and was similar to ‘Maxifort’ plants grown at the standard in-row spacing ($P<0.05$; Table 3.3).

The benefit of grafting with ‘Maxifort’ rootstock was not significantly penalized by reducing planting densities. In the 2008 trial, an additional treatment was included whereby plants were grown at half the standard planting density (5,980 plants/ha). Interestingly, the main effect of plant spacing on fruit yield was not significant (Table 3.1). Furthermore, grafted plants grown at the half-rate planting density (5,980 plants/ha) had similar per hectare crop yield compared to the nongrafted ones grown at the standard plant spacing (Table 3.3). Similarly to 2007, the ‘Maxifort’ plants grown at the intermediate plant spacing (7,970 plants/ha) had higher fruit yield compared to the nongrafted CP at the standard spacing, but unlike 2007, this difference was not statistically significant (Table 3.3).

In addition to ‘Beaufort’ and ‘Maxifort’, ‘Big Power, and ‘RST-04-105-T’ were evaluated in the open-field system to determine the effect of grafting with inter-specific rootstocks on crop yield in non-infested soils for organic heirloom tomato production. The main effect of rootstock on yield was significant ($P<0.05$; Table 3.1) and the impact of rootstock on crop yield is shown on Table 3.4. Plants grafted onto ‘Beaufort’ and ‘Maxifort’ had significantly higher fruit yield and mean size compared to nongrafted plants ($P<0.05$; Table 3.4). ‘Big Power’, and ‘RST-04-105-T’ had intermediate crop yields and were statistically similar to nongrafted plants as well as those grafted onto
‘Beaufort’ and ‘Maxifort’ rootstocks (Table 3.4). The effect of rootstock on fruit size was significant ($P<0.05$; Table 3.4), but fruit number was not affected (Table 3.1).
CONCLUSIONS

In this study, grafting the heirloom cultivar, ‘Cherokee Purple’ (CP), with interspecific rootstocks was highly advantageous in an organic production setting. In both years, fruit yield of CP was increased significantly when grafted onto ‘Beaufort’ and ‘Maxifort’ rootstocks compared to the nongrafted CP in both the high tunnels and the open-field ($P<0.05$). In these trials, cropping land had not been in tomato production previously, and there was no disease pressure from any economically-important root-infecting pathogens (O’Connell et al., 201X). This represents the first report of consistent yield effects in heirloom tomato production in non-infested soils as a result of grafting with ‘Beaufort’ or ‘Maxifort’ rootstocks.

When ‘German Johnson’ was grafted onto ‘Maxifort’ and grown in the open-field, the yield benefit of grafting was not consistent and when a benefit existed, it occurred late in the year (Rivard and Louws, 2008). Our results with CP showed that the most consistent yield benefit across all systems and in both years occurred during the second harvest period and the benefit was statistically significant 100 days after planting. One explanation for the difference seen between the previous report (Rivard and Louws, 2008) and this study is the nongrafted/scion cultivar and this could be an important factor. Further evidence can be seen in a more recent report with CP, and ‘Beaufort’ and ‘Maxifort’ had a similar beneficial effect on total and marketable fruit yield across two years even though disease pressure was much more severe in the first year of the trials (Rivard et al., 2010). These studies suggest that further research which explores the
relationship between scion cultivar and the benefit of grafting with inter-specific rootstocks will be valuable for the successful implementation of grafting in the US.

Although the two rootstocks had statistically similar yields, ‘Beaufort’ performed slightly better in 2008 whereas ‘Maxifort’ did better in 2007, particularly in the high tunnels. It’s not clear why these trends occurred or if they were related to environmental conditions. In 2007, growing conditions were hot compared to 2008 (O’Connell et al., 201X), and high temperatures and the effects of thermal and moisture stress could have been further amplified in the tunnel growing system. One indication of this trend is the reduced yield of the nongrafted plants grown in the high tunnels compared to the open-field in this particular year. Grafting tomatoes with inter-specific rootstocks has been effective at reducing thermal stress (Abdelmageed and Gruda, 2009; Rivero, Ruiz, and Romero, 2003a, 2003b; Venema et al., 2008), but little information regarding the rootstocks ‘Maxifort’ or ‘Beaufort’ for this trait is available. Similarly, the 2007 growing season included a severe drought and although the trials were under drip irrigation, the plants may have been undergoing water stress during this particular year. Grafting with ‘Beaufort’ and other inter-specific rootstocks has been shown to dramatically affect root hair number as well as root length and density (Oztekin et al., 2009) and this effect could have given the plants grafted onto ‘Beaufort’ and ‘Maxifort’ rootstocks an advantage over nongrafted plants during periods of water stress.
A second objective of this study was to determine if grafting has a differential effect within open-field and high tunnel growing systems and to understand how this may be related to the extended harvest season within the high tunnels. If a differential benefit of grafting occurs and is related to the extended growing season, then the system*rootstock interaction would not exist 100 DAP and would be present in the “systems” analysis. This hypothesis was not supported by our data and our conclusions show that the benefit of grafting is similar in both the open-field and high tunnel systems, regardless of the season length. In a previous study where ‘Big Red’ scion was grafted onto ‘Heman’ and ‘Primavera’ rootstocks, there was no benefit of grafting in either system (Khah et al., 2006). As discussed previously, these contrasting conclusions may be due to the different nongrafted/scion cultivars used.

An important finding in the open-field system is that the planting density of grafted heirloom tomatoes can be reduced while maintaining high crop productivity. This is the first report that addresses this question using a stake-and-weave training system in the open-field. Similar to previous findings with high tunnel and greenhouse systems (Besri, 2003; Kubota et al., 2008), grafted plants could be grown at one-half the standard planting density of nongrafted CP without penalizing crop yield. Additionally, plants grown at the intermediate density in 2007 had greater yield than the nongrafted crop at the standard planting density ($P<0.05$). It should be noted that the objective of this study was not to describe an optimum planting density for grafted heirloom plants.
Additional studies that seek to address this question from both an economic and production perspective will be of value to heirloom tomato growers in the US.

Grafting will be an important component of organic heirloom tomato production for growers who wish to reduce disease outbreaks and increase crop yield. In high tunnels, the ability to rotate or move the structure may be difficult and soilborne diseases are best-handled preventatively (Beckerman, 2004; Chellemi, 2002; Pottorff and Panter, 2009). Grafting with resistant rootstocks is an excellent tool for managing soilborne pathogens without soil fumigants (King et al., 2008; Kubota et al., 2008) and the rootstocks utilized in this study are resistant to several economically-important soilborne diseases (Rivard and Louws, 2008; Rivard et al., 2008; 2010). The results of this report indicate that rootstocks such as ‘Beaufort’ and ‘Maxifort’ can also increase yield in non-infested soils and ‘Big Power’ and ‘RST-04-105-T’ had intermediate yields.

A clear question is whether or not this yield benefit makes grafting economically feasible without disease pressure. If this were the case, then grafting could be utilized to preventatively reduce crop losses by soilborne pathogens. This advantage could be particularly useful in high tunnel production systems where crop rotation intervals are often reduced (Beckerman, 2004). The integration of grafting and high tunnels to manage foliar and soilborne diseases will be instrumental for successful organic heirloom tomato production in the US.
<table>
<thead>
<tr>
<th>Analysis</th>
<th>Year(s)</th>
<th>Parameter</th>
<th>Main plot</th>
<th>Sub plot</th>
<th>P-values&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Main</th>
<th>Sub</th>
<th>Main*Sub</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 day factorial</td>
<td>2007</td>
<td>Fruit wt.</td>
<td>System</td>
<td>Rootstock</td>
<td>0.005, &lt;0.001</td>
<td>0.005</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>Fruit wt.</td>
<td>System</td>
<td>Rootstock</td>
<td>0.026, 0.018</td>
<td>0.026</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>Fruit no.</td>
<td>System</td>
<td>Rootstock</td>
<td>0.004, 0.001</td>
<td>0.004</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>Fruit no.</td>
<td>System</td>
<td>Rootstock</td>
<td>0.006, 0.324</td>
<td>0.006</td>
<td>0.324</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>Mean size</td>
<td>System</td>
<td>Rootstock</td>
<td>0.143, 0.272</td>
<td>0.143</td>
<td>0.272</td>
<td>0.172</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>Mean size</td>
<td>System</td>
<td>Rootstock</td>
<td>0.119, 0.002</td>
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<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Systems factorial</td>
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<td>Fruit wt.</td>
<td>System</td>
<td>Rootstock</td>
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<td></td>
<td>&lt;0.001</td>
<td>0.384</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>Fruit wt.</td>
<td>System</td>
<td>Rootstock</td>
<td>0.006, 0.002</td>
<td>0.006</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>Fruit no.</td>
<td>System</td>
<td>Rootstock</td>
<td>0.244, &lt;0.001</td>
<td>0.244</td>
<td>&lt;0.001</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>Fruit no.</td>
<td>System</td>
<td>Rootstock</td>
<td>0.002, 0.105</td>
<td>0.002</td>
<td>0.105</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>Mean size</td>
<td>System</td>
<td>Rootstock</td>
<td>0.270, 0.088</td>
<td>0.270</td>
<td>0.088</td>
<td>0.390</td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>Mean size</td>
<td>System</td>
<td>Rootstock</td>
<td>0.280, &lt;0.001</td>
<td>0.280</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>% Mktble</td>
<td>System</td>
<td>Rootstock</td>
<td>0.362, 0.368</td>
<td>0.362</td>
<td>0.368</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>% Mktble</td>
<td>System</td>
<td>Rootstock</td>
<td>0.002, 0.084</td>
<td>0.002</td>
<td>0.084</td>
<td>0.247</td>
</tr>
<tr>
<td>Field intervals&lt;sup&gt;w&lt;/sup&gt;</td>
<td>2007</td>
<td>Fruit wt.</td>
<td>Rootstock</td>
<td>Period</td>
<td>0.039, &lt;0.001</td>
<td>0.039</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>Fruit wt.</td>
<td>Rootstock</td>
<td>Period</td>
<td>0.075, &lt;0.001</td>
<td>0.075</td>
<td>&lt;0.001</td>
<td>0.022</td>
</tr>
<tr>
<td>Tunnel intervals&lt;sup&gt;w&lt;/sup&gt;</td>
<td>2007</td>
<td>Fruit wt.</td>
<td>Rootstock</td>
<td>Period</td>
<td>0.170, &lt;0.001</td>
<td>0.170</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>Fruit wt.</td>
<td>Rootstock</td>
<td>Period</td>
<td>0.053, &lt;0.001</td>
<td>0.053</td>
<td>&lt;0.001</td>
<td>0.277</td>
</tr>
<tr>
<td>Field spacing&lt;sup&gt;y&lt;/sup&gt;</td>
<td>2007</td>
<td>Fruit wt.</td>
<td>Spacing</td>
<td>Rootstock</td>
<td>0.008, 0.008</td>
<td>0.008</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>Fruit wt.</td>
<td>Spacing</td>
<td>Rootstock</td>
<td>0.230, 0.023</td>
<td>0.230</td>
<td>0.023</td>
<td>0.493</td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>Fruit no.</td>
<td>Spacing</td>
<td>Rootstock</td>
<td>0.197, 0.241</td>
<td>0.197</td>
<td>0.241</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>Fruit no.</td>
<td>Spacing</td>
<td>Rootstock</td>
<td>0.056, 0.056</td>
<td>0.056</td>
<td>0.056</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2007</td>
<td>Mean size</td>
<td>Spacing</td>
<td>Rootstock</td>
<td>0.231, 0.055</td>
<td>0.231</td>
<td>0.055</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2008</td>
<td>Mean size</td>
<td>Spacing</td>
<td>Rootstock</td>
<td>0.120, 0.013</td>
<td>0.120</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td>Field rootstocks&lt;sup&gt;s&lt;/sup&gt;</td>
<td>Combined</td>
<td>Fruit wt.</td>
<td>Rootstock</td>
<td>Year</td>
<td>0.017, 0.003</td>
<td>0.017</td>
<td>0.003</td>
<td>0.299</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>Fruit no.</td>
<td>Rootstock</td>
<td>Year</td>
<td>0.035, 0.035</td>
<td>0.035</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>Mean size</td>
<td>Rootstock</td>
<td>Year</td>
<td>0.018, 0.159</td>
<td>0.018</td>
<td>0.159</td>
<td>0.376</td>
</tr>
</tbody>
</table>

<sup>1</sup> All P-values were generated using the MIXED procedure (SAS 9.1; SAS Institute, Cary, NC).
<sup>2</sup> Represents fruit yield that had accumulated 100 days after planting in open-field and high tunnel systems.
<sup>3</sup> Represents final fruit yield (116 and 146 days after planting in the open-field and high tunnels, respectively).
<sup>4</sup> Cumulative yield data from the systems factorial study was collected into five arbitrary harvest intervals and analyzed using the REPEATED statement.
<sup>5</sup> Represents treatments that were trialed in the open-field but not in the high tunnels.
<sup>6</sup> In-row spacing of grafted and nongrafted plants was manipulated to reflect standard, two-thirds, and one-half rate planting densities.
<sup>7</sup> Two additional rootstocks were trialed in the open-field.
Table 3.2. Main effects* of grafting on total fruit yield in 2007 and 2008.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Rootstock</th>
<th>Total yield (t/ha)</th>
<th>Fruit no. (10^3/ha)</th>
<th>Fruit size (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 day factorial*</td>
<td>Non-grafted</td>
<td>67.7 y</td>
<td>61.2 a</td>
<td>220.5 y</td>
</tr>
<tr>
<td></td>
<td>Beaufort</td>
<td>90.5 z</td>
<td>77.5 b</td>
<td>294.5 z</td>
</tr>
<tr>
<td></td>
<td>Maxifort</td>
<td>95.9 z</td>
<td>73.8 ab</td>
<td>299.0 z</td>
</tr>
<tr>
<td>Systems factorial*</td>
<td>Non-grafted</td>
<td>90.2 a</td>
<td>96.1 y</td>
<td>472.2 *</td>
</tr>
<tr>
<td></td>
<td>Beaufort</td>
<td>117.0 b</td>
<td>131.2 z</td>
<td>592.3 *</td>
</tr>
<tr>
<td></td>
<td>Maxifort</td>
<td>133.8 b</td>
<td>126.2 z</td>
<td>597.3 *</td>
</tr>
<tr>
<td>Field spacing*</td>
<td>Non-grafted</td>
<td>122.7 a</td>
<td>73.2 y</td>
<td>499.0</td>
</tr>
<tr>
<td></td>
<td>Maxifort</td>
<td>146.1 b</td>
<td>86.0 z</td>
<td>496.6</td>
</tr>
</tbody>
</table>

* Combined data from high tunnel and open-field system as well as various planting densities in the open-field. Data were analyzed by the MIXED procedure and Tukey's mean separation test was used (α=0.05). Values followed by a * indicate significant main*subplot interaction.

† The results from individual analyses can only be compared within each set of results/rootstocks for a given year.

‡ Non-grafted 'Cherokee Purple' were compared to 'Cherokee Purple' grafted onto 'Beaufort' and 'Maxifort' rootstocks

§ Represents fruit yield that had accumulated 100 days after planting in open-field and high tunnel systems.

¶ Represents final fruit yield (116 and 146 days after planting in the open-field and high tunnels, respectively).

* In-row spacings of grafted and nongrafted plants were manipulated to reflect standard, two-thirds, and one-half rate planting densities.
Table 3.3. Effects\(^w\) of grafting and planting density on total fruit yield in the open-field\(^x\).

<table>
<thead>
<tr>
<th>Rootstock(^z)</th>
<th>Plants/ha(^y)</th>
<th>Year</th>
<th>Total yield (t/ha)</th>
<th>Fruit no. (10^7/ha)</th>
<th>Fruit size (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nongrafted</td>
<td>11,960</td>
<td>2007</td>
<td>125.3 a</td>
<td>565.6 a</td>
<td>229 a</td>
</tr>
<tr>
<td>Nongrafted</td>
<td>7,970</td>
<td>2007</td>
<td>120.2 a</td>
<td>432.5 a</td>
<td>279 ab</td>
</tr>
<tr>
<td>Maxifort</td>
<td>11,960</td>
<td>2007</td>
<td>146.6 b</td>
<td>526.7 a</td>
<td>286 ab</td>
</tr>
<tr>
<td>Maxifort</td>
<td>7,970</td>
<td>2007</td>
<td>145.6 b</td>
<td>466.4 a</td>
<td>317 b</td>
</tr>
<tr>
<td>Nongrafted</td>
<td>11,960</td>
<td>2008</td>
<td>77.3 y</td>
<td>389.7 yz</td>
<td>198 y</td>
</tr>
<tr>
<td>Nongrafted</td>
<td>7,970</td>
<td>2008</td>
<td>75.6 y</td>
<td>348.3 xyz</td>
<td>217 yz</td>
</tr>
<tr>
<td>Nongrafted</td>
<td>5,980</td>
<td>2008</td>
<td>66.6 y</td>
<td>293.0 x</td>
<td>229 z</td>
</tr>
<tr>
<td>Maxifort</td>
<td>11,960</td>
<td>2008</td>
<td>97.7 z</td>
<td>419.1 z</td>
<td>232 z</td>
</tr>
<tr>
<td>Maxifort</td>
<td>7,970</td>
<td>2008</td>
<td>81.8 yz</td>
<td>352.8 xyz</td>
<td>234 z</td>
</tr>
<tr>
<td>Maxifort</td>
<td>5,980</td>
<td>2008</td>
<td>78.5 yz</td>
<td>322.9 xy</td>
<td>243 z</td>
</tr>
</tbody>
</table>

\(^w\) Data were analyzed by the MIXED procedure and Tukey’s mean separation test was used (α=0.05). Values followed by the same letter are not significantly different.

\(^x\) Open-field plants were grown with stake-and-weave trellis system and harvested up until 116 days after planting.

\(^z\) Nongrafted ‘Cherokee Purple’ were compared to ‘Cherokee Purple’ grafted onto ‘Maxifort’ rootstocks.

\(^y\) In-row spacing of grafted and nongrafted plants was manipulated to reflect standard, two-thirds, and one-half rate planting densities.
Table 3.4. Main effects\(^x\) of grafting on fruit yield in the open-field\(^y\).

<table>
<thead>
<tr>
<th>Rootstock(^z)</th>
<th>Total yield (t/ha)</th>
<th>Fruit no. (10^5) /ha</th>
<th>Fruit size (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-grafted</td>
<td>101.3 a</td>
<td>477.7 a</td>
<td>213 a</td>
</tr>
<tr>
<td>Beaufort</td>
<td>124.8 b</td>
<td>510.8 a</td>
<td>245 ab</td>
</tr>
<tr>
<td>Maxifort</td>
<td>122.1 b</td>
<td>472.9 a</td>
<td>259 b</td>
</tr>
<tr>
<td>Big Power</td>
<td>118.2 ab</td>
<td>477.7 a</td>
<td>247 ab</td>
</tr>
<tr>
<td>RST-04-105-T</td>
<td>118.0 ab</td>
<td>496.6 a</td>
<td>239 ab</td>
</tr>
</tbody>
</table>

\(^x\) Combined data from 2007 and 2008 were analyzed by the MIXED procedure and Tukey’s mean separation test was used \((\alpha=0.05)\). Values followed by the same letter are not significantly different.

\(^y\) Open-field plants were grown with stake-and-weave trellis system and harvested up until 116 days after planting.

\(^z\) Nongrafted ‘Cherokee Purple’ were compared to ‘Cherokee Purple’ grafted onto four inter-specific rootstocks.
Figure 3.1. Fruit yield of nongrafted ‘Cherokee Purple’ and CP grafted onto ‘Beaufort’ and ‘Maxifort’ rootstocks that had accumulated 100 days after planting in the open-field and high tunnel systems in 2007. Mean fruit size (A), fruit number (B), and total fruit weight (C) data were analyzed using the MIXED procedure and the error bars represent the standard error of the mean.
Figure 3.2. Fruit yield of nongrafted ‘Cherokee Purple’ and CP grafted onto ‘Beaufort’ and ‘Maxifort’ rootstocks that had accumulated 100 days after planting in the open-field and high tunnel systems in 2008. Mean fruit size (A), fruit number (B), and total fruit weight (C) data were analyzed using the MIXED procedure and the error bars represent the standard error of the mean.
Figure 3.3. Fruit yield of nongrafted ‘Cherokee Purple’ and CP grafted onto ‘Beaufort’ and ‘Maxifort’ rootstocks grown in the open-field and high tunnel systems in 2007. Mean fruit size (A), fruit number (B), and total fruit weight (C) data were analyzed using the MIXED procedure and the error bars represent the standard error of the mean.
Figure 3.4. Fruit yield of nongrafted ‘Cherokee Purple’ and CP grafted onto ‘Beaufort’ and ‘Maxifort’ rootstocks grown in the open-field and high tunnel systems in 2008. Mean fruit size (A), fruit number (B), and total fruit weight (C) data were analyzed using the MIXED procedure and the error bars represent the standard error of the mean.
Figure 3.5. Total fruit yield of nongrafted ‘Cherokee Purple’ and CP grafted onto ‘Beaufort’ and ‘Maxifort’ rootstocks collected during each arbitrary harvest interval. A * indicates the presence of a significant rootstock effect at a given harvest interval (P<0.05). A) 2007 open-field. B) 2008 open-field. C) 2007 high tunnel. D) 2008 high tunnel.
LITERATURE CITED


ACKNOWLEDGEMENTS

The authors wish to sincerely thank the Center for Environmental Farming Systems (CEFS), North Carolina Department of Agriculture and Consumer Services (NCDA&CS), and the Southeastern Plant Environment Laboratory. Special thanks to Josh Moore, Carolyn Lowry, Amanda McWhirt, Ryan Faulk, Seth Avis, the CEFS internship and apprenticeship programs, as well as Steve Moore, Ken Fager, and the NCDA staff located at the Cherry Research Station (Goldsboro, NC). Thanks to Peter Ojiambo (NCSU) for statistical assistance and comments by E.L. Davis and H. D. Shew. Rootstock seed donated by De Ruiter Seeds, Rijk Zwaan Seeds, and DP Seed Co. Funding provided by Southern Region SARE LS06-193 and USDA-NIFA 2007-51106-03794.
CHAPTER FOUR

The following report

Grafting tomato to manage bacterial wilt (caused by

Ralstonia solanacearum) in the southeastern US

by

C.L. Rivard, S. O’Connell, M.M. Peet, R.M. Welker, and F.J. Louws

was prepared for submission to

Plant Disease

The American Phytopathological Society

St. Paul, MN
ABSTRACT

Rivard, C.L., S. O’Connell, M.M. Peet, R.M. Welker, and F.J. Louws. 201X. Grafting tomato to manage bacterial wilt (caused by *Ralstonia solanacearum*) in the southeastern US. Plant Dis. XX:XXX.

Bacterial wilt (BW), caused by *Ralstonia solanacearum*, causes severe losses to tomato (*Solanum lycopersicum*) in the southeastern US and grafting may be an effective strategy for managing this disease. However, *R. solanacearum* maintains considerable diversity and little information is known regarding the efficacy of commercially-available rootstocks to reduce BW incidence in the US. In this study, plants grafted onto ‘Dai Honmei’ and ‘RST-04-105-T’ rootstocks had lower AUDPC values compared to non- and self-grafted plants (*P<0.05*). Across three locations, final BW incidence of non- and self-grafted plants ranged from 61% to 100%. In contrast, BW incidence of the plants grafted with commercially-available rootstocks ranged from no symptomatic plants to 65% disease incidence. ‘Dai Honmei’ conferred high resistance at two locations in western NC and intermediate resistance at the site in eastern NC. ‘RST-04-105-T’ conferred high resistance in two locations, but had similar BW AUDPC values to the non-grafted plants at the third. Total fruit yield was significantly increased by grafting onto resistant rootstocks (*P<0.05*). Regression analysis indicated that yield was correlated with BW AUDPC values (R² ranged from 0.4048 to 0.8034) and the use of resistant rootstocks allowed for crop productivity to be maintained even in soils that were naturally-infested with *R. solanacearum*. 
INTRODUCTION

Bacterial wilt (BW), caused by *Ralstonia solanacearum*, can be a serious problem for tomato growers that have infested soils due to the complex biology of the pathogen, severity of the disease, and lack of effective management strategies (3, 13). *R. solanacearum* infects tomato roots through wounds or natural openings, colonizes the xylem, and produces extracellular polysaccharides that clog the vascular tissue (15). Ultimately, this prevents upward water movement through the stem and total collapse of the plant may occur in 2-5 days (26). This disease can cause severe yield losses (3, 13) and result in growers abandoning fields from tomato production due the presence of the pathogen.

*Ralstonia solanacearum* has a host range of more than 50 plant families (21) and maintains considerable diversity worldwide (13, 21, 37, 41). *R. solanacearum* attacks several economically-important solanaceous crops including tomato, pepper, potato, eggplant, and tobacco (13). *R. solanacearum* is also metabolically versatile, surviving in diverse habitats such as water, soil, and latently in infected plants (13). It is also easily-dispersed and can move into non-infested areas through water, soil, infected plant material, and by mechanical means (3, 6, 13, 21, 26, 35). The world population of *R. solancearum* is separated into four biovars (14) and three races (3), and has been recently classified into phylotypes and sequevars (9). In the southeastern US, Phylotype II, Sequevar 7 (formerly biovar 1) is of greatest importance and occurs endemically (9, 26).
Control of BW is difficult for tomato growers in the southeastern US, particularly for those that are not able to rotate out of tomato production for extended time periods. The wide host range of *R. solanacearum* restricts rotational options, and effective crop rotation programs in heavily-infested soils may require multiple years out of tomato production (23). Even soil fumigants have little success against this pathogen (7, 8), and vertical movement of *R. solanacearum* may allow for the bacteria to quickly re-colonize fumigated beds (35).

Host resistance could be a useful option for managing BW, but numerous challenges exist for plant breeders. Resistance to BW in tomato is quantitative, and is strongly influenced by environmental conditions such as soil temperature, pH, and moisture (37). Even when resistance is effective, it is typically strain-specific (12, 41), and the diversity of pathogenic strains of *Ralstonia* has led to the development of resistant lines that are not durable over diverse geographic regions (36). Another issue that has been problematic for tomato breeders is that small fruit size is linked to resistance to BW (29, 40, 41). One way to circumvent this problem is by grafting cultivars that have good fruit production characteristics onto BW resistant rootstocks.

Grafting has been utilized to manage BW in tomato crops worldwide (11, 24, 25, 30, 39), and has been proposed recently in the US (22, 33). Breeding lines CRA 66 and Hawaii 7996 were found to significantly reduce BW incidence in naturally-infested soils in North Carolina (33). Although these findings are important for the implementation of rootstock breeding programs in the US, they do not provide information that is readily-
useable for growers who face this devastating disease. Rootstocks are available from commercial seed companies that provide resistance to BW (32). However, their ability to confer resistance against native *R. solanacearum* strains in the US is currently unknown.

Because grafting could be a valuable tool for tomato growers in the southeastern US, it is of critical importance to evaluate commercially-available rootstocks for resistance to strains of *R. solanacearum* present in the southeastern US. Therefore, the objectives of this study were (i) to evaluate commercially-available tomato rootstocks for their ability to reduce bacterial wilt and other soilborne disease incidence in naturally-infested soils, and (ii) to determine if any conferred resistance results in increased fruit yield.
MATERIALS AND METHODS

Trials were established in one location in 2007 and 2008, and two different locations in 2009. All trials were conducted in a randomized complete block design with four replications. As is typical for the region, raised-bed plasticulture was employed with a stake-and-weave plant training system (16). Water was provided by drip irrigation and fertility was supplied according to standard on-farm protocols (see below).

BW disease ratings were performed throughout the course of the growing season. Disease incidence was recorded based on the presence *R. solanacearum* and visible symptoms of BW on the host. Symptoms of bacterial wilt included a complete loss of turgor and total plant collapse (26). Stem segments of wilted plants (2.5 cm) were excised, surface sterilized, and used to isolate *R. solanacearum* from infected plants on Kelman's TZC semi-selective medium (20). Excised stems of symptomatic plants were also subjected to an Rs Immunostrip (Agdia, Elkhart, IN) assay to verify the presence of *R. solanacearum*.

**Transplant production and grafting:** All grafted and non-grafted tomato transplants were produced at the Southeastern Plant Environment Laboratory located at North Carolina State University (Raleigh, NC; http://www.ncsu.edu/phytotron/). ‘Celebrity’ (Harris Seeds; Rochester, NY) and ‘Mountain Fresh’ (Harris Seeds; Rochester, NY) were used as the scion cultivar for rootstock treatments and for non- and self-grafted
controls. These are hybrid cultivars with determinate growth patterns commonly used for open-field production, and have no known resistance to bacterial wilt. 'Celebrity' was used in the Sampson county trials and 'Mountain Fresh' was utilized at the Jackson and Henderson county locations.

Commercially-available rootstock cultivars, ‘RST-04-104-T’ (DP Seeds; Yuma AZ), Dai Honmei (Asahi Industries Co.; Saitama, Japan), TMZQ702 (Sakata Seed; Yokohama, Japan), and one currently unreleased line, ‘DR-BW-NCS2’ (De Ruiter Seeds; Bergschenhoek, The Netherlands) were utilized for rootstock treatments in these studies. In addition to the rootstock-specific cultivars tested for resistance to BW, a grape-type tomato, ‘Sweet Olive’ (Johnny’s Selected Seeds; Winslow, ME), was used as rootstock in the Sampson county trial in 2008.

In the Sampson and Jackson county trials, a self-grafted treatment was included where the scion variety was grafted onto its original root system to account for any grafting/healing effects. The Japanese tube grafting technique was utilized for all grafted plants (32). Rootstock and scion seedling stems were severed and held together using a silicon clip. Once grafted, the plants were immediately moved into a "healing chamber" where humidity and light conditions are manipulated to promote graft union formation (32).

**Sampson County trials:** Field trials were conducted in 2007 and 2008 at a commercial organic farm located in the Coastal Plain region in Sampson County, NC (34.6138069 N,
The soil type was a Chipley sand (pH = 6.0). This farm is certified organic and tomatoes were taken out of open-field production due to recurring crop failures from severe BW pressure (28). In 2007, the grafting treatments included ‘Celebrity’ grafted onto 'Dai Honmei', and 'RST-04-105-T' rootstocks in addition to non- and self-grafted ‘Celebrity’. In 2008, two rootstock treatments were added, ‘DR-BW-NCS2’ and ‘Sweet Olive’.

The trials were conducted in two 34 m rows (2 blocks per row) in 2007 and two 51 m rows (2 blocks per row) in 2008, respectively. In both years, the grafting treatments were randomly assigned to 3.7 m plots within each of the four blocks; treatments were re-randomized in 2008. Seven plants were located within each plot and cultural methods were consistent with on-farm tomato production. Plants were spaced at 53 cm within the row, and rows were 1.5 m apart. Pre-plant nitrogen was supplied through cover crop incorporation and supplemental feathermeal applications of 167 Kg N/ha.

The Sampson County experiments were initiated on 20 Apr 2007 and 22 Apr 2008. In 2007, harvesting was carried out on: 18, 22, 26 and 30 Jun; 4, 10, 14, 19, 26, and 31 Jul; 3 and 8 Aug. In 2008, harvesting was conducted on: 23 and 28 Jun; 4, 9, 14, 22, and 26 Jul. All tomato fruit were harvested and graded as marketable or non-marketable based upon on-farm standards, and fruit weight and number were recorded for each grade.
**Jackson County trial:** A field trial was conducted in 2009 at a commercial farm located in the western mountain region in Jackson County, NC (35.4227163 N, 83.3082677 W). The soil type was a Braddock clay loam (pH = 6.5). This field had recent repeated crop failures due to severe BW pressure in one particular area (approx. 0.5 ha in 2008). The grafting treatments included 'Mountain Fresh' grafted onto 'Dai Honmei', and 'RST-04-105-T' rootstocks in addition to non- and self-grafted 'Mountain Fresh'.

The Jackson County trial was conducted on two 29 m rows (2 blocks per row). The grafting treatments were randomly assigned to 3.2 m plots within each of the four blocks. Seven plants were located within each plot and cultural methods were consistent with on-farm tomato production. Plants were spaced at 46 cm within the row, and rows were 1.5 m apart. Blended preplant fertilizer (8-3-20) was applied at 672 kg/ha, and two Ca(NO₃)₂ fertigation supplements were supplied at 67 kg/ha on 30 Jul and 17 Aug.

The Jackson County trial was initiated on 21 Jul 2009. Fruit was harvested on: 23 and 29 Sep; and 6 and 15 Oct. All tomato fruit were harvested and graded as marketable or non-marketable based upon on-farm standards, and fruit weight and number were recorded for each grade. On the final date, all fruit larger than 5 cm were harvested and recorded.

**Henderson county trial:** A field trial was conducted in 2009 at the Mountain Horticultural Crops Research and Extension Center (http://www.agr.state.nc.us/...
research/mhcrs.htm) in Henderson County, NC (35.4267596 N, 82.5574439 W). The soil type was a Elsinboro loam (pH = 6.4). The grafting treatments included 'Mountain Fresh' grafted onto 'Dai Honmei', 'RST-04-105-T', ‘DR-BW-NCS2’, and ‘TMZQ702’ rootstocks in addition to non-grafted ‘Mountain Fresh’.

The trial was conducted on twenty 15 m rows (1 plot per row) and 12 plants of each treatment were centrally-located within the rows at 46 cm in-row spacing. The grafting treatments were randomly assigned to each row within a given block, and rows were 1.5 m apart. Blended preplant fertilizer (13-34-10) was applied at 672 kg/ha, and six Ca(NO₃)₂ fertigation supplements were supplied at 16.8 kg N/ha on 24 June; 1, 15 and 29 Jul; and 5 and 12 Aug.

The Henderson County trial was initiated on 9 Jun 2009. Fruit was harvested on: 27 Aug; 3, 10, and 17 Sep. All fruit were harvested and graded as marketable or non-marketable based upon the appearance of fruit shriveling, blossom-end rot, insect damage, severe fruit cracking, or size (i.e. too small), and fruit weight and number were recorded for each grade. On the final date, all fruit larger than 5 cm were harvested and recorded.

**Statistical analysis:** Data collected from all research trials were analyzed similarly, but independently. An area under the disease progress curve (AUDPC) was calculated (38) based on the incidence of BW or any other documented soilborne diseases. AUDPC values and yield data were analyzed using the MIXED procedure and an estimate of the
means was generated using LSMEANS (SAS 9.1; SAS Institute, Cary, NC). Where significant treatment effects occurred, Tukey’s mean comparison test was utilized (SAS 9.1; SAS Institute, Cary, NC).

In order to determine the effect of AUDPC values on marketable and total fruit yield, the CORR procedure (SAS 9.1; SAS Institute, Cary, NC) was performed using the data gathered from all treatments within a given trial. The REG procedure (SAS 9.1; SAS Institute, Cary, NC) was also carried out to determine an $R^2$ value that described the variance in total fruit yield which was accounted for by variance in the corresponding AUDPC values.
RESULTS

Sampson county trials: In 2007, BW (caused by R. solanacearum) was evident, particularly in the late period of the season, and the effect of grafting with inter-specific rootstocks on BW AUDPC values was significant (P<0.01; Table 4.1). The BW epidemic began 75 days after planting among non- and self-grafted plants and the final BW disease incidence was 100% and 82%, respectively (Fig 4.1A). ‘Celebrity’ plants grafted onto ‘Dai Honmei’ rootstocks had intermediate levels of BW throughout the season and the final BW incidence was 50%. ‘RST-04-105-T’ rootstocks conferred high resistance and plants grafted onto this rootstock showed no symptoms of BW (Fig 4.1A). The AUDPC values reflected a similar trend suggesting that over the course of the season, ‘Dai Honmei’ and ‘RST-04-105’ rootstocks provided intermediate and high levels of protection, respectively, against BW (P<0.05; Fig 4.1B).

In 2008, Ralstonia solanacearum was found in several plants throughout the field plots. However, southern blight (caused by S. rolfsii) was the dominant disease problem in this particular year. The first observation of southern blight occurred 50 days after planting and 14 days later (64 days after planting) 50% and 57% of non- and self-grafted plants had been killed by S. rolfsii, respectively (Fig 4.2A). Among all of the plants that died throughout the course of the season, R. solanacearum was identified using Rs immunostrips in 25% and 29% of the non- and self-grafted plants and from 7% of the plants grafted with ‘DR-BW-NCS2’ rootstock.
The effect of grafting on southern blight AUDPC values was significant ($P<0.01$; Table 4.1). The three inter-specific rootstocks as well as the grape-type rootstock reduced disease incidence over time (Fig 4.2B). ‘Dai Honmei’, ‘RST-04-105-T, and ‘DR-BW-NCS2’ rootstocks reduced the level of disease in the field compared to non- and self-grafted plants ($P<0.05$; Fig 4.2B). ‘Sweet Olive’ had intermediate southern blight AUDPC values and was not significantly different than the non- or self-grafted controls (Fig 4.2B).

In 2007, total and marketable fruit yield were correlated with BW AUDPC values ($P<0.05$; $R^2_{_{\text{Tot yield}}} = 0.4048$) and similar results were seen with southern blight AUDPC values in 2008 ($P<0.001$; $R^2_{_{\text{Tot yield}}} = 0.7255$). In 2008, a higher amount of variation in yield was accounted for by the AUDPC values compared to 2007 resulting in a higher $R^2$ value determined from the 2008 data. This contrast suggests that disease levels among all the treatments in the 2007 trial had a lesser effect on fruit yield than in 2008. In 2008, at least 50% of the non- and self-grafted plants had been killed by southern blight 64 days after planting (Fig 4.2A) whereas in 2007, similar levels of BW were not seen until 88 days after planting (Fig 4.1A).

The reduced incidence of BW and southern blight among the grafted plants in conjunction with the correlation between AUDPC values indicate that the resistance seen amongst the rootstocks translated to increased fruit yield. The effect of grafting on total fruit yield was significant in both years ($P<0.05$; Table 4.1). In 2007, ‘Dai Honmei’ increased total fruit yield by 30% compared to non-grafted plants and the 78% yield
increase seen as a result of grafting with ‘RST-04-105-T’ was statistically significant
($P<0.05$; Table 4.2). Similarly to 2007, plants grafted with ‘RST-04-105-T’ in 2008 had
the highest total fruit yield and were significantly different than the self-grafted
‘Celebrity’ plants ($P<0.05$; Table 4.3). Total fruit yield among the ‘RST-04-105-T’
rootstocks in 2008, however, was not significantly different than the yield of plants
grafted onto ‘Dai Honmei’, and ‘DR-BW-NCS2’ rootstocks, and yield increases as a result
of grafting onto these inter-specific rootstocks ranged from 133% to 226% (Table 4.3).

**Jackson county trial:** In the on-farm trial in Jackson county, the BW epidemic began 36
days after planting and BW AUDPC values were highly impacted by grafting ($P<0.001$;
Table 4.1). BW incidence of the non-grafted plants was 54% 56 days after planting and
the final BW incidence was 86% and 82%, respectively (Fig 4.3A). ‘Mountain Fresh’
plants grafted onto ‘RST-04-105-T’ rootstocks had intermediate levels of BW
throughout the season and the final BW incidence was 50%. ‘Dai Honmei’ rootstock
conferred high resistance and plants grafted onto this rootstock showed no symptoms
of BW (Fig 4.3A). The AUDPC values reflect a similar trend suggesting that over the
course of the season, ‘Dai Honmei' and ‘RST-04-105’ rootstocks provided high and
intermediate levels of protection, respectively ($P<0.05$; Fig 4.3B).

In the trial in Jackson county, marketable and total fruit yield were correlated
with BW AUDPC values ($P<0.001$; $R^2_{\text{Tot yield}} = 0.6426$). The large amount of variance in
total fruit yield among the treatments that was accounted for by BW AUDPC indicates
that BW had a particularly important role on fruit production. In this trial, the epidemic was initiated early, and at first fruit harvest (64 days after planting), non- and self-grafted plants had 64% and 43% BW disease incidence, respectively (Fig 4.3A).

The effect of grafting on total and marketable fruit production was highly significant ($P<0.01$; Table 4.1) and yield increases ranging from 31% to 103% can be seen with the use of the two rootstocks (Table 4.4) Plants grafted onto ‘Dai Honmei’ had the highest yields and total fruit yield was increased by 102% compared to the non-grafted plants ($P<0.05$; Table 4.4). ‘RST-04-105-T’ had 31% higher total fruit yield than the non-grafted plants, but this effect was not significant ($P<0.05$; Table 4.4).

**Henderson county trial:** In the Henderson county trial, the BW epidemic began 30 days after planting and at first fruit harvest 90% of the non-grafted plants had been killed by *Ralstonia solanacearum* (Fig 4.4A). Grafting had a significant effect on BW AUDPC values ($P<0.001$; Table 4.1). Among the four rootstocks tested in the Henderson county trial, ‘Dai Honmei’ and ‘DR-BW-NCS2’ had the lowest levels of disease throughout the season and final BW incidence of these two rootstocks was 13% and 4%, respectively (Fig 4.4A). This reduction in BW incidence resulted in decreased BW AUDPC values compared to the non-grafted plants ($P<0.05$; Fig 4.4B). ‘RST-04-105-T’ and ‘TMZQ702’ delayed the onset of disease, but final BW incidence was 65% and 63% (Fig 4.4A), and BW AUDPC values were not significantly different than the non-grafted plants ($P<0.05$; Fig 4.4B).
 Marketable and total fruit yield were highly correlated to BW AUDPC and a large portion of the variability in total fruit yield was accounted for by BW AUDPC values in this trial ($P<0.0001; R^2_{\text{Tot yield}} = 0.8034$). Similarly to the Jackson county location, the higher $R^2$ value indicates that the levels of disease throughout the year were important in explaining the differences seen in fruit yield. In this trial, the disease epidemic was initiated earlier than in the other three trials and the rapid increase in BW incidence that occurred prior to first harvest may have been particularly important (Fig 4.4A)

The effect of grafting on total and marketable fruit production was highly significant ($P<0.01; \text{Table 4.1}$) and yield increases ranged from 68% to 332% among the four rootstocks tested as compared to the non-grafted plants (Table 4.5). Plants grafted with ‘DR-BW-NCS2’ rootstocks had the highest fruit yield and total and marketable fruit production was 260% and 332% higher than non-grafted plants, respectively ($P<0.05; \text{Table 4.5}$). ‘Celebrity’ plants with ‘Dai Honmei’ rootstocks had 231% higher total fruit yield than the non-grafted plants ($P<0.05; \text{Table 4.5}$). ‘RST-04-105-T’ and ‘TMZQ702’ 128% and 72% higher fruit yield than the non-grafted ‘Mountain Fresh’ plants, but was not significantly different ($P<0.05; \text{Table 4.5}$).
CONCLUSIONS

In heavily infested soils, *R. solanacearum* has the ability to severely reduce tomato fruit yield, and grafting with resistant rootstocks provides an effective management tool that growers can utilize to manage yield losses caused by this pathogen. In our study, marketable and total fruit yield were correlated with BW AUDPC values and ‘RST-04-105-T’, ‘Dai Honmei’, and ‘DR-BW-NCS2’ rootstocks had reduced AUDPC values compared to non- and self-grafted plants across multiple locations and years. Interestingly, ‘RST-04-105-T’ and ‘Dai Honmei’ rootstocks conferred high and intermediate resistance levels in Sampson County, respectively, whereas the opposite trend was seen in the Jackson and Henderson County trials.

The diversity of *Ralstonia* strains has led to the development of resistant lines that are not durable over diverse geographic regions (36), and based on the results of this study, it may be hypothesized that significant diversity exists across the different geographic locations utilized in this report. In Sampson County tobacco and vegetable crops have historically been grown, and *R. solanacearum* has been indigenous in this area as indicated by early reports of BW in the literature (5, 19). In contrast BW is an emerging issue in the mountain tomato growing region of western NC, like Jackson and Henderson Counties (Louws, personal observations). Recent studies suggest that new strains of *R. solanacearum* that are entering the US may be poorly characterized (27) and a new strain has been of significant interest in Florida (18). It’s not clear how these findings are related to the results in this study, but future work that seeks to
characterize *R. solanacearum* strains and identify the impact of the pathogen population on the efficacy of tomato rootstocks will be of value to tomato growers in the US.

Although the quantitative level of resistance was not equal across the various geographic locations, the results of this study suggest that ‘Dai Honmei’ and ‘RST-04-105-T’ can provide protection from severe crop failures due to quantitative resistance. Other tomato rootstocks have shown efficacy worldwide against BW (11, 24, 25, 30, 39), but little information is available regarding the specific rootstocks utilized in these trials. In a recent preliminary report from the Eastern shore of VA, ‘RST-04-105-T’ performed similarly to the Henderson County trial (10).

The partial resistance to BW seen in these studies could complement other integrated pest management strategies to further reduce BW incidence under severely-infested conditions. For example, the plant activator acibenzolar-S-methyl reduced BW among moderately-resistant cultivars (31). Other strategies that show quantitative efficacy against BW (1, 2, 4, 17) could be combined with grafting to further reduce the incidence of BW in severely-infested soils.

In this study, the rootstocks that were resistant to BW also showed intermediate resistance to southern blight in the 2008 Sampson County trial. The southern blight epidemic occurred unexpectedly and these results highlight the ability of inter-specific rootstocks to withstand severe pressure from multiple plant pathogens. The rootstocks evaluated in this study were selected to reduce BW, but also provided protection from southern blight (*P*<0.05). Recently, ‘Big Power’, ‘Beaufort’, and ‘Maxifort’ rootstocks
were reported to confer resistance to southern blight (34). Although the results pertaining to southern blight in this study are preliminary in nature, they may be important to growers who wish to deploy rootstocks to manage BW, particularly if they have had previous problems with southern blight (S. rolfsii). The rootstocks that were resistant to southern blight (34) did not confer resistance to BW (Rivard and Louws, unpublished data). Therefore, other rootstocks may be more suitable for growers in the US that do not have soils infested with R. solanacearum, but have problems with S. rolfsii.

This study provides the first report of commercially-available rootstock cultivars that growers in the southeastern US can utilize to manage BW, and grafting with interspecific rootstocks provides a conduit for rapid deployment of these traits. Quantitative resistance traits are often widely-known, but their utilization is problematic, and this paradigm is particularly acute in the case of bacterial wilt (29, 40, 41). This disease causes severe losses to growers that have infested soils and grafting provides an effective management strategy to reduce BW incidence and subsequent crop loss.
Table 4.1. Effects$^w$ of grafting on yield and AUDPC$^x$ at four tomato trials performed from 2007-2009.

<table>
<thead>
<tr>
<th>Location</th>
<th>Year</th>
<th>Marketable fruit yield</th>
<th>Total fruit yield</th>
<th>AUDPC$^x$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(t/ha)</td>
<td>Size (g)</td>
<td>No. (10$^3$/ha)</td>
</tr>
<tr>
<td>Samspon Co.</td>
<td>2007</td>
<td>0.072</td>
<td>0.030</td>
<td>0.286</td>
</tr>
<tr>
<td>Samspon Co.</td>
<td>2008</td>
<td>0.003</td>
<td>0.023</td>
<td>0.007</td>
</tr>
<tr>
<td>Jackson Co.</td>
<td>2009</td>
<td>0.008</td>
<td>0.432</td>
<td>0.055</td>
</tr>
<tr>
<td>Henderson Co.</td>
<td>2009</td>
<td>&lt;0.001</td>
<td>0.003</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^w$ The listed $P$-values were determined using proc GLM (SAS 9.1; SAS Institute; Cary, NC).

$^x$ Area under the disease progress curve.

$^y$ AUDPC was calculated based on the incidence of bacterial wilt (caused by *Ralstonia solanacearum*).

$^z$ AUDPC was calculated based on the incidence of southern blight (caused by *Sclerotium rolfsii*).
Table 4.2. Tomato fruit yield of grafted and non-grafted 'Celebrity' at Sampson County\(^x\) in 2007

<table>
<thead>
<tr>
<th></th>
<th>Marketable fruit yield(^z)</th>
<th>Total fruit yield(^z)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(t/ha) Size (g) No. (10(^3)/ha)</td>
<td>(t/ha) Size (g) No. (10(^3)/ha)</td>
</tr>
<tr>
<td>Non-grafted</td>
<td>39.1 a 168 ab 232.5</td>
<td>62.4 a 157 ab 397.4</td>
</tr>
<tr>
<td>Self-grafted</td>
<td>43.9 ab 180 b 248.1</td>
<td>67.9 a 166 ab 412.7</td>
</tr>
<tr>
<td>Dai Honme(^ix)</td>
<td>47.4 ab 184 ab 257.1</td>
<td>81.3 ab 177 ab 490.3</td>
</tr>
<tr>
<td>RST-04-105(^ix)</td>
<td>64.7 b 202 b 319.1</td>
<td>110.8 b 192 b 581.6</td>
</tr>
</tbody>
</table>

\(x\) Under moderate disease pressure from bacterial wilt (*Ralstonia solanacearum*).
\(y\) Data were analyzed using proc GLM and the means were compared with Tukey's mean separation test (\(\alpha=0.05\)). Values followed by the same letter are not significantly different.
\(z\) Inter-specific rootstocks grafted with 'Celebrity' scion.

Table 4.3. Tomato fruit yield of grafted and non-grafted 'Celebrity' at Sampson County\(^w\) in 2008

<table>
<thead>
<tr>
<th></th>
<th>Marketable fruit yield(^w)</th>
<th>Total fruit yield(^w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(t/ha) Size (g) No. (10(^3)/ha)</td>
<td>(t/ha) Size (g) No. (10(^3)/ha)</td>
</tr>
<tr>
<td>Non-grafted</td>
<td>13.4 a 201 65.9 a</td>
<td>19.3 ab 176 105.4 ab</td>
</tr>
<tr>
<td>Self-grafted</td>
<td>10.4 a 192 53.2 a</td>
<td>14.8 a 171 85.2 a</td>
</tr>
<tr>
<td>Dai Honme(^ix)</td>
<td>31.2 ab 235 131.4 ab</td>
<td>44.9 ab 214 209.1 c</td>
</tr>
<tr>
<td>RST-04-105(^ix)</td>
<td>43.7 b 242 177.5 b</td>
<td>61.3 b 235 253.1 c</td>
</tr>
<tr>
<td>DR-BW-NCS2(^ix)</td>
<td>32.2 ab 234 135.8 ab</td>
<td>45.0 ab 215 208.7 c</td>
</tr>
<tr>
<td>Sweet Olive(^ix)</td>
<td>17.4 a 199 87.1 a</td>
<td>31.3 ab 172 180.4 bc</td>
</tr>
</tbody>
</table>

\(w\) Under severe disease pressure from southern blight (*S. rolfsii*) and bacterial wilt (*R. solanacearum*).
\(x\) Data were analyzed using proc GLM and the means were compared with Tukey's mean separation test (\(\alpha=0.05\)). Values followed by the same letter are not significantly different.
\(y\) Inter-specific rootstocks grafted with 'Celebrity' scion.
\(z\) Cherry-type rootstock grafted with 'Celebrity' scion.
### Table 4.4. Tomato fruit yield of grafted and non-grafted 'Mountain Fresh' at Jackson County<sup>x</sup> in 2009

<table>
<thead>
<tr>
<th></th>
<th>Marketable fruit yield&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Total fruit yield&lt;sup&gt;y&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(t/ha)</td>
<td>Size (g)</td>
</tr>
<tr>
<td>Non-grafted</td>
<td>13.0 a</td>
<td>114</td>
</tr>
<tr>
<td>Self-grafted</td>
<td>14.6 a</td>
<td>119</td>
</tr>
<tr>
<td>Dai Honmei&lt;sup&gt;z&lt;/sup&gt;</td>
<td>26.5 b</td>
<td>126</td>
</tr>
<tr>
<td>RST-04-105&lt;sup&gt;z&lt;/sup&gt;</td>
<td>17.1 ab</td>
<td>111</td>
</tr>
</tbody>
</table>

<sup>x</sup> Under moderate disease pressure from bacterial wilt (*Ralstonia solanacearum*).

<sup>y</sup> Data were analyzed using proc GLM and the means were compared with Tukey’s mean separation test (α=0.05). Values followed by the same letter are not significantly different.

<sup>z</sup> Inter-specific rootstocks grafted with 'Mountain Fresh' scion.

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### Table 4.5. Tomato fruit yield of grafted and non-grafted 'Mountain Fresh' at Henderson County<sup>x</sup> in 2009

<table>
<thead>
<tr>
<th></th>
<th>Marketable fruit yield&lt;sup&gt;y&lt;/sup&gt;</th>
<th>Total fruit yield&lt;sup&gt;y&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(t/ha)</td>
<td>Size (g)</td>
</tr>
<tr>
<td>Non-grafted</td>
<td>11.0 a</td>
<td>191</td>
</tr>
<tr>
<td>Dai Honmei&lt;sup&gt;z&lt;/sup&gt;</td>
<td>40.6 ab</td>
<td>256</td>
</tr>
<tr>
<td>RST-04-105&lt;sup&gt;z&lt;/sup&gt;</td>
<td>30.1 ab</td>
<td>221</td>
</tr>
<tr>
<td>DR-BW-NCS2&lt;sup&gt;z&lt;/sup&gt;</td>
<td>47.5 b</td>
<td>256</td>
</tr>
<tr>
<td>TMZQ702&lt;sup&gt;z&lt;/sup&gt;</td>
<td>18.5 ab</td>
<td>223</td>
</tr>
</tbody>
</table>

<sup>x</sup> Under severe pressure from bacterial wilt (*Ralstonia solanacearum*).

<sup>y</sup> Data were analyzed using proc GLM and the means were compared with Tukey’s mean separation test (α=0.05). Values followed by the same letter are not significantly different.

<sup>z</sup> Inter-specific rootstocks were grafted with 'Mountain Fresh' scion.
Figure 4.1 – Mean bacterial wilt incidence (A) and AUDPC (B) of non- and self-grafted 'Celebrity' tomato as well as 'Celebrity' grafted onto 'Dai Honmei' and 'RST-04-105-T' rootstocks at the Sampson County trials in 2007. AUDPC data were analyzed by Tukey’s mean comparison test ($P = 0.05$).
Figure 4.2 – Mean southern blight incidence (A) and AUDPC (B) of non- and self-grafted ‘Celebrity’ tomato as well as ‘Celebrity’ grafted onto ‘Dai Honmei’, ‘RST-04-105-T’, ‘DR-BW-NCS2’, and ‘Sweet Olive’ rootstocks at the Sampson County trials in 2008. AUDPC data were analyzed by Tukey’s mean comparison test ($P = 0.05$).
Figure 4.3 – Mean bacterial wilt incidence (A) and AUDPC (B) of non- and self-grafted 'Mountain Fresh' tomato as well as 'Mountain Fresh' grafted onto 'Dai Honmei' and 'RST-04-105-T' rootstocks at the Jackson County trials in 2009. AUDPC data were analyzed by Tukey’s mean comparison test ($P = 0.05$).
Figure 4.4 – Mean bacterial wilt incidence (A) and AUDPC (B) of non- and self-grafted ‘Mountain Fresh’ tomato as well as ‘Mountain Fresh’ grafted onto ‘Dai Honmei’, ‘RST-04-105-T’, ‘DR-BW-NCS2’, and ‘TMZQ702’ rootstocks at the Henderson County trials in 2009. AUDPC data were analyzed by Tukey’s mean comparison test ($P = 0.05$).
LITERATURE CITED


ACKNOWLEDGEMENTS

The authors wish to sincerely thank the NCSU Phytotron staff as well as Monica Schiessl, Amanda McWhirt, Ryan Faulk, and Seth Avis for technical assistance. Special thanks to Christine Bredenkamp (NC Coop. Ext. Serv.) and Peter Ojjiambo (NCSU) as well as our on-farm collaborators: Stefan Hartmann, Kent Cochran, and the NCDA staff at the Mountain Horticultural Crops Research and Extension Center (Mills River, NC). Rootstock seed donated by De Ruiter Seeds, DP Seeds, Asahi Industries Co, and Sakata Seed. Funding provided by Southern Region SARE LS06-193 and USDA NIFA2007-51106-03794.
SUMMARY AND CONCLUDING REMARKS

With the phaseout of methyl bromide and increased market for organic produce, tomato growers in the US require soilborne disease management strategies that reduce reliance on chemical fumigation (13). Additionally, the arrival of high tunnels into US tomato production (10) and the unique set of challenges that these structures offer provide a new opportunity for the use of grafted tomato plants (56). Grafting could be particularly useful for heirloom and/or organic growers as these markets can provide high economic returns for growers that are able to cater to local and direct-markets (20, 33). Through collaborative inter-disciplinary research, twenty-nine replicated field experiments were conducted in North Carolina (NC) and Pennsylvania (PA) from 2005-2009 to identify the utility of grafting with inter-specific rootstocks under organic and conventional practices. These trials were implemented to evaluate the role of rootstocks to reduce disease incidence as well as investigate cultural methods such as plant spacing, pruning, fertility, and growing system. In many of these cases, trials were repeated over years and across locations and the details of published work are provided in the preceding chapters. This essay will attempt to summarize findings from the NC and PA studies and integrate these data with those available from the literature to gain a holistic perspective on the value of grafting for US tomato production. In the second section of the paper, important aspects concerning the future of tomato grafting in the US and worldwide are discussed.
GRAFTING AS A SUSTAINABLE DISEASE MANAGEMENT TOOL

Tomato production in the US, and particularly in the southeast, is limited significantly by the prevalence and severity of soilborne pathogens. The mild winter climate in the southeast coupled with the history of planting multiple solanaceous crops (e.g. tobacco, tomato, pepper, eggplant, potato) can lead to severe losses from soilborne plant pathogens. High tunnels quickly gained popularity with small growers in the Northeastern US in the early 1990’s (90), and have increased in popularity throughout the US, particularly for tomato production (10). Crop rotation in these systems is often reduced or eliminated due to economic constraints (6, 63) and soil fumigants are becoming problematic due to the associated regulatory and economic costs (46, 54).

Host resistance is a sustainable disease management strategy that has been utilized in conventional and organic production systems. Diseases like fusarium wilt (caused by *Fusarium oxysporum* f.sp. *lycopersici*) can be managed relatively easily with this method as major resistance genes are available in tomato, and these resistance genes have been introgressed into commercial cultivars (92) as well as inter-specific rootstocks (66). The severe selection pressure that deployment of major resistance genes applies to the pathogen population can result in pathogen races that are able to overcome resistance (50). In the case of *F. oxysporum* f.sp. *lycopersici*, this fungal plant pathogen population has developed three races (45), and major resistance genes are available for all of these in both hybrid cultivars (92) as well as rootstocks.
If major resistance genes are available in modern tomato hybrids for a pathosystem in question and growers are able to easily switch cultivar, the ability to reduce diseases by grafting may be of little consequence. However, many growers are limited to particular cultivars due to their production characteristics (e.g. greenhouse and specialty cultivars) or for marketing purposes. Heirloom tomato growers represent the latter of these groups, and grafting may be particularly useful for this niche (66). An often over-looked detail concerning grafting of heirloom cultivars is that because these varieties are highly susceptible to diseases, any hybrid that carries major resistance genes against soilborne diseases could be useful as rootstocks (66). For example, ‘Mtn Fresh Plus F1’ carries resistance genes against *F. oxysporum* f.sp. *lycopersici*, races 1 and 2 (92), and this hybrid cultivar may be useful for heirloom growers that have soils infested with this pathogen.

Although major gene resistance is known for verticillium wilt, *Verticillium dahliae* (race 2) rapidly developed in tomato and no known resistance to race 2 of the pathogen exists (62). This disease is primarily managed in NC as well as throughout the Mid-Atlantic growing region with soil fumigants. Soil fumigation is able to delay the onset of the disease such that yields can be captured to provide adequate economic returns (81). Ultimately, however, the pathogen population is not eradicated from infested soils and the production system becomes dependent on fumigation.

In other pathosystems like bacterial wilt (caused by *Ralstonia solanacearum*) and southern blight (*Sclerotium rolfsii*) of tomato, major gene resistance is not known in
Solanum spp., but quantitative resistance traits are available. Resistance to R. solanacearum has been documented among wild Solanum spp., but fruit size is tightly linked to resistance, and breeders have had difficulty providing adequately resistant varieties with large, marketable fruit (58, 87, 88). This paradigm has been evident in the case of bacterial wilt for decades and this disease was one of the primary driving forces that led to the adoption of tomato grafting in Asia in the mid-20th century (39, 57).

Grafting will play an important role in management of root- and stem-infecting pathogens as this technology can be quickly deployed without causing significant changes in farming operations. Vegetable grafting is common in Asia and Europe (36, 38, 39), and rootstock-specific hybrids have been developed for tomato with resistance to soilborne pathogens. However, the successful adoption of this technology in the US requires a critical examination of rootstocks to target site-specific soilborne pathogens. Field trials were performed throughout NC and PA from 2005-2009 to examine the utility of commercially-available and “pipeline” rootstocks to reduce losses to soilborne pathogens or yield losses caused by these organisms. The summarized results of these trials are shown in Table 1 and the specific pathosystems trialed are discussed below.

**Bacterial wilt**: Bacterial wilt of tomato was one of the first pathosystems where grafting was utilized worldwide (36, 39, 57) and the severity of this disease coupled with the pathogen’s ability to survive in the soil could make this technology very attractive to growers in the US. Symptoms of bacterial wilt include severe wilting and
ultimately results in total collapse of the plant (48). Typically, plant mortality results in acute yield losses. Furthermore, *R. solanacearum* is metabolically diverse, is able to survive on weeds and saprophytically in the soil, and moves easily into non-infested areas (such as fumigated beds) through water and during flooding events (26, 35).

Bacterial wilt continues to plague tomato growers in central and eastern NC and growers will abandon fields from tomato production due to infested soils.

As previously mentioned, grafting has been adopted successfully worldwide to reduce bacterial wilt incidence (22, 42, 47, 60, 82). However, resistance to *R. solanacearum* is typically strain-specific (24, 88), and the diversity of *Ralstonia* has led to the development of resistant lines that are not durable over diverse geographic regions (77). Therefore, it is crucial to evaluate rootstocks for resistance to bacterial wilt at local and regional geographic scales. In NC, breeding lines ‘CRA 66’ and ‘Hawaii 7996’ showed excellent resistance to bacterial wilt when utilized as rootstocks in severely infested fields (67). As rootstocks have become commercially-available in the US, several of these have been tested in NC (71) and elsewhere (18) and show promise for growers that have fields infested with *R. solanacearum*.

Although grafting shows significant promise to manage bacterial wilt, it should be noted that the complex diversity of this pathogen and the nature of resistance will require continued evaluation of rootstocks. In NC, ‘RST-04-105-T’ was highly resistant to the native *R. solanacearum* population on the eastern part of the state whereas this rootstock performed poorly in western NC (71) and the Eastern Shore of VA (18).
similar manner, ‘Dai Honmei’ had intermediate resistance in Eastern NC, but performed well at two locations in western NC (71). Taken together, these results suggest that the diversity that exists in *R. solanacearum* strains throughout the southeastern US will impact the quantitative efficacy of tomato rootstocks for management of this pathogen.

In most cases, the rootstocks in these trials showed partial resistance to bacterial wilt, and the cost of grafted transplants could deter growers from adopting grafting. Grafting adds between $0.46 and $1.12 per plant (70) and any plant loss that occurs could result in undesirable economic effects. Therefore, the partial resistance to bacterial wilt seen in these studies should be complemented by other integrated pest management strategies to further reduce disease incidence under severely-infested conditions. For example, the plant activator acibenzolar-S-methyl reduced bacterial wilt among moderately-resistant cultivars (64). Other strategies like crop rotation, solarization, biofumigation, or treatment with bio-pesticides (1, 3, 13, 30) could be combined with grafting to further reduce the incidence of bacterial wilt in severely-infested soils and provide better economic returns.

The last area where grafting may be of interest to tomato growers and plant breeders is in the case of one specific strain of *R. solanacearum*, race 3 biovar 2 (r3b2). *R. solanacearum* (r3b2) does not currently exist in the US, but has been introduced through imported ornamental planting stock several times (80). This strain is particularly aggressive, survives in cool climates, and could pose a significant threat to tomato crops (80). ‘Hawaii 7996’ is a viable source of resistance for *R. solanacearum*.
and the development of rootstocks resistant to this strain is an important opportunity for future work.

**Southern blight:** An unexpected finding during recent grafting trials in NC was that many inter-specific rootstocks confer resistance to southern blight (*caused by Sclerotium rolfsii*) (69). *S. rolfsii* has a broad host range (>600 species) (16), and diseases caused by this pathogen are especially rampant in areas of the southern US where temperatures are sufficiently high to permit the growth and survival of the fungus (65). Southern blight results in rapid and permanent wilt of all aboveground parts resulting in severe yield losses (49). Since *S. rolfsii* has a wide host range and can persist on virtually all types of crop residues, rotation to non-hosts is not practical (65). Additionally, its ability to colonize crop residues can be problematic for growers who utilize cover crops or no-till production and this disease is seen frequently on organic farms in NC (Rivard and Louws, unpublished data).

In a recent report, ‘Big Power’, ‘Beaufort’, and ‘Maxifort’ rootstocks provided high resistance to southern blight (69), and similar results were seen with several others including ‘RST-04-105-T’ and ‘Dai Honmei’ in a preliminary report (55). Generally, partial resistance was observed suggesting that resistance is quantitative, and may be due to multi-genic host resistance traits. *S. rolfsii* produces oxalic acid and other cell wall-degrading enzymes as it infects its host (65). Due to the activity of this toxin, resistance to infection by *S. rolfsii* is typically related to calcium concentration in
host tissues (52, 65, 89). Rootstocks can have a significant effect on leaf tissue nutrient concentrations, but few studies have looked at calcium within the stem. In one report, ‘Beaufort’ rootstock elevated calcium concentration in the epigeous biomass (stem, leaf, and fruit tissue) of grafted tomato plants (41). It’s not clear if calcium is mediating resistance in the inter-specific rootstocks that have shown resistance to southern blight, but this may be an important question for future research.

**Root-knot nematodes:** Root-knot nematodes (*Meloidogyne* spp.) are obligate endoparasites that infect a wide number of crop plants and cause severe losses in yield (4, 59). In the US, *M. incognita* (the southern root-knot nematode) is most prevalent, although *M. hapla* occurs in northern climates (59). Low populations of root-knot nematodes may cause little economic damage, but severe infestations result in root-galling, wilting, nutritional deficiencies, and stunting, and poor yield (59). Soil fumigants like methyl bromide, methyl iodide, and 1,3 – dichloropropene are effective at reducing *M. incognita* populations (27), but cannot be used in organic production. Crop rotation is can be utilized to reduce RKN populations (31, 79). However, the wide host range of *M. incognita* makes development of rotations schemes difficult without resistant crops.

A major resistance gene, *Mi*, is available for commercial hybrids (91) and has been bred into several inter-specific tomato rootstocks (14). Similar to most major resistance genes, *Mi*-mediated resistance results in a hypersensitive response upon
detection by the host and the nematode is not able to establish a suitable feeding site (91). Grafting with rootstocks that carry the Mi gene has been highly effective worldwide (14, 19, 28, 34, 36) and shows promise in the US (44, 69). In NC, grafting tomato with ‘Big Power’ rootstock was more effective at reducing root-knot nematode galling index and root-knot nematode soil populations than fumigation with 1,3-dichloropropene, a fumigant nematicide (69).

An unexpected result of recent work with tomato grafting (14, 44, 69) is that some inter-specific rootstocks that carry Mi do not have the phenotypical characteristics of plants that confer Mi-mediated resistance. Several rootstocks, including ‘Beaufort’ and ‘Maxifort’ maintained intermediate resistance despite verification of Mi by molecular markers (14, 44, 69). Interestingly, in these studies crop yields were not penalized and Lopez-Perez et al. (44) suggested host tolerance was being conferred. Currently, it is difficult to be certain that tolerance is occurring and this could be an interesting subject for future research. However, it should also be noted that careful recommendations should be made to growers who wish to utilize inter-specific rootstocks in soils infested with root-knot nematodes (Meloidogyne spp.).

A key question that results from these findings is to what extent can root-knot nematode damage occur on rootstocks before crop loss is evident? The population dynamics of root-knot nematodes can significantly alter yield penalties in tomato (5) and future work that seeks to establish nematode thresholds relating to inter-specific rootstocks would be invaluable for rootstock recommendations. Oftentimes, growers
are forced to manage multiple diseases in a single field. Rotation between rootstocks that show high and partial resistance against root-knot may be an effective way of keeping the pathogen population level low while simultaneously managing other issues. Further work is needed to define the thresholds that lead to economic damage in rootstocks.

**Grafting for increased vigor and yield:** Although grafting with inter-specific rootstock provides an excellent deployment method for utilizing host resistance, rootstocks could also provide increases in crop yield through added vigor. There are two primary reasons why added vigor may be a particularly important factor when considering grafting as a disease management strategy. First, if rootstocks are available (or can be developed) that provide enough yield benefit to make grafting economically feasible for growers without infested soils, then it can be utilized preventatively to reduce pathogen outbreaks. Root diseases are best managed in this way (13), and if grafting were economically feasible under little or no disease pressure (56), then it could be used to reduce risk in tomato production systems. There are reports in the literature where grafting under little/no disease pressure provides a yield benefit (43, 61, 83). However, this question is complicated and could potentially be affected by rootstock, scion/nongrafted cultivar, growing system, cultural methods, environment, etc.

Recent findings from NC indicate that in the case of indeterminate heirloom cultivars, scion choice may be particularly important. ‘Cherokee Purple’ showed
consistent yield benefits from ‘Beaufort’ and ‘Maxifort’ rootstocks in open-field and high tunnel production systems with non-infested soils (68). In contrast, fruit yield of ‘German Johnson’ was increased with ‘Maxifort’ in only one of three field trials under little/no disease pressure and the ‘Robusta’ provided no benefit at the two locations where it was tested (67). An important question for future research will be the interaction that occurs between rootstock and scion vigor and how it impacts crop yield under little disease pressure.

The second point that should be made concerning plant vigor is that tomato growers could potentially adopt reduced planting densities to lessen per ha cost of grafted transplants subsequently making this disease management practice more economically feasible. Greenhouse operations in the US have adopted a “twin-leader” production system (38) and high tunnel operations in Morocco have adopted similar methods (7). Recent work from NC showed that in the open-field, in-row plant spacings can be manipulated without penalizing crop yields compared to nongrafted plants at standard plant spacings (68). Although only trialed for one season, similar results were seen in PA with the determinate hybrid cultivar, ‘BHN 589’ (23). These results suggest that plant spacing will be a particularly important issue for growers as it can have a dramatic impact on the per ha cost of using grafted plants.

**Economics of grafting:** One of the most complex question that still remains for those interested in grafting in the US pertains to the economics of this practice. In a recent
report, the added cost of using grafted transplants ranged from $0.46 to $1.12 per plant, and nongrafted transplants were $0.13 to $0.76 per plant (70). A similar estimate was also provided by Kubota et al. (38). These represent a relatively wide range of costs that a grower may encounter when implementing grafting on the farm.

Although it is likely the cost of grafted transplants may go down in the US as this technology becomes more widely-adopted, it is important to note that these transplants will always be considerably more expensive than nongrafted ones. In the case study from NC, nongrafted plants were produced for $0.13 per plant whereas the cost of rootstock seed alone was $0.20 per plant (38). Similarly, other costs such as the materials (e.g. soil, trays, etc.) required for production of scion material as well as grafting labor and materials (e.g. clips, tools, healing chamber, etc) will demand a much higher price for grafted plants compared to nongrafted ones. However, preliminary results suggest that in cases where disease pressure is high, the added costs required during plant propagation can translate to substantial economic benefits during fruit production (55).

Under little disease pressure or in pathosystems where crop losses are less acute, the economic benefit of grafting becomes less clear for similar reasons to those stated above. A simpler approach could be taken by growers who wish to consider the economic relevance of grafting by identifying the amount of revenue generated per plant. Based on the results of the recent report (70), tomato fruit growers would need to capture at least $0.46 to $1.12 more income per plant in order to offset the cost of
grafting, and a higher benefit would be ideal in order to gain from the added investment of grafted transplants.

Per plant revenue is driven by the yield as well as the selling price of fruit. Fruit yield is highly variable across cultivars and growing systems (e.g. open-field vs high tunnel). During trials with grafted and nongrafted plants in NC and PA, per plant yields ranged from 10 lbs (4.5 kg) to 40 lbs (18 kg) of fruit per plant. Even more variable, however, is the selling price of tomato fruit across the diverse markets in the US, and this may be a more important consideration for growers. In 2009, wholesale tomato prices were as low $0.32/lb ($0.15/kg) during peak times of the season (2) whereas heirloom fruit sold through direct markets can be priced as high as $7/lb ($3.18/kg) (33). Ultimately, grafting will only be economically feasible for tomato growers that not only see a yield benefit, but can also capture enough return from that benefit to use grafted plants in a profitable way.
THE FUTURE OF TOMATO GRAFTING IN THE US

Currently, there is significant interest in grafting for the US and this will continue to grow as tomato growers look to find alternative ways of managing soilborne disease and increasing crop yields. Grafting is also particularly useful worldwide in protected cultivation and high tunnels (68), and the increased popularity of these systems in the US (10) will most likely lead to further adoption of grafted plants. However, several challenges exist in the realm of tomato grafting and opportunities for work in this area are substantial. This section of the essay will focus on current needs and some potentially fruitful avenues within grafting research and technology delivery that could be vital to a comprehensive future exploration of tomato grafting for the US and worldwide.

The critical need for technology transfer: Interest in tomato grafting in the US has expanded rapidly in the last decade (36, 38), but few growers are currently producing grafted plants. Exceptions to this include several small and organic growers in the northeast where tomato grafting has been utilized in greenhouse and high tunnel production (V. Grubinger, personal communication). As the result of a number of recent workshops and other activities provided by research and extension personnel at several land-grant universities, growers throughout the country are attempting to propagate plants on their own. Since 2007, the author of this essay has been contacted...
by people not only in NC, but also CA, FL, GA, IN, LA, MI, MO, NY, PA, and WA seeking assistance with grafted transplant propagation.

The need for technology delivery in this regard is of utmost importance and was cited as one of the dominant issues that currently restricts the utilization of grafted plants in the US in a recent review by Kubota et al. (38). Grafted transplant propagation is difficult for a multitude of reasons. Namely, the grafting procedure requires skilled labor. More importantly, management of newly grafted plants is complex, and environmental conditions within the healing chamber can favor disease if contamination occurs. Additionally, rootstock and scion seedlings must have similar stem diameters on the day of grafting, and variability in germination period and subsequent growth of the plants can make grafted transplant production problematic. Compared to non-grafted tomato transplant production, this system is remarkably complex. In order for growers to fairly evaluate the utilization of grafted plants for fruit production purposes, extension efforts focused on grafted transplant propagation should be expanded in the US.

Opportunities exist within tomato grafting to engage grower clientele, and this could advance the way that extension and outreach is performed in the US. There is a strong need to develop an internet-based digital media outlet for growers to learn more about not only the benefits of grafting, but also the technique that is required. Traditional means of digital communication such as instructional videos, web seminars,
discussion blogs, etc. could be extraordinarily useful for delivery of information regarding tomato rootstocks as well as grafting technique.

The delivery of grafting-related information is an excellent way to engage growers and document impact, particularly with young farmers. There are a number of web-based grower groups (e.g. GardenWeb: Growing Tomatoes Forum; http://forums.gardenweb.com/forums/tomato/ ) and commodity organizations (e.g. Carolina Farm Stewards Assoc, etc.) that currently have a substantial web presence, and typically these groups cater to young and developing farmers, oftentimes growing organically or without the use of pesticides. Not only could interactions with these groups foster the delivery of applicable information related to this emerging technology (grafting), but a centralized digital library would be vital to connect with this community and secure a productive relationship between extension and clientele.

Other digital tools may also become available to help growers make research-based decisions, and these could be used as models for future extension needs. For example, a recent report showed how the per plant costs of grafted and non-grafted transplants accumulated over time (70). This type of analysis could be adapted into a digital applet that would allow growers to input typical seed, labor, and material costs and provide an estimated cost of grafted and non-grafted plants at their own facility. Previous discussion indicated the importance of per plant revenue for growers who are considering the implementation of grafted plants. Similar economic applets could be designed that would help growers make practical decisions regarding grafting based on
their typical average yields and selling price of fruit. These types of tools are simple in design, but carry significant weight with growers as they provide valuable and readily-accessible information.

**Grafted tomato propagation:** Upon initiation of tomato grafting research at NC State University, it was assumed that the most important data that could be gathered for growers related to tomato grafting in the US would focus on the utilization of grafted plants. Based on the available research (55, 67-69, 71), it appears evident that grafting will be beneficial for some growers in the US and future work may find additional utility in production with grafted plants. However, it should also be noted that more research is required to develop grafted propagation methods for tomato.

There are scattered reports in the literature of cultural methods that can be conducted to increase grafting success. Low levels of light and wind within the healing chamber increased the success rate of grafted tomatoes (53). An obvious result from experience with tomato grafting in NC is that growers need more specific information related to healing chamber management. Oftentimes, it is speculated that poor success is correlated with heat stress or improper humidity during the healing phase. However, there is no data currently available to predict what temperatures are too hot or precisely where humidity levels should be for optimum healing.

Another productive avenue for future research in grafted tomato propagation is the use of hormones not only to increase grafting success, but also to reduce
adventitious root formation during the healing process. Adventitious roots from susceptible scions could lead to infection by soilborne pathogens in the field (66), and in newly grafted plants they seem to be induced by stress and/or excess relative humidity (Rivard and Louws, unpublished data). Other practical research for propagators could include investigations into healing chamber thermal dynamics as well as the effect of leaf-pruning or chemical applications to reduce water stress during healing.

There are a variety of questions related to grafted tomato propagation that are economic in nature as well. In a recent case study from Pennsylvania, the indirect costs of producing a second crop for scion material constituted 30% of the added cost of grafting (70). These costs could be reduced through innovative production techniques. Micrografting has been recently introduced into the arena of tomato grafting this technique uses micro-propagated scion grafted onto 3 week-old rootstock seedlings (21). For growers unable to carry out this type of procedure, indirect costs could be reduced by growing scion varieties in high density seedling trays and sown after the rootstock crop to ensure proper stem diameters. In this way, grafted transplants could be produced for a lesser price. Similarly, grafting robots have been proposed worldwide as a way to reduce costs of grafted transplants (38, 40), and manual grafting accounted for 14% to 24% of the added cost of grafting in a recent report (70).
**Grafting to reduce foliar diseases:** An unexplored area of research that could be particularly fruitful involves the use of tomato rootstocks to reduce foliar diseases. Rootstocks could mediate damage by foliar pathogens indirectly by making shoot tissues less susceptible to infection by foliar pathogens through enhanced nutrient uptake or other metabolic effects. Plant nutrition plays an important role on basal plant defense mechanisms (15), and nitrogen availability can have effects on foliar diseases of tomato like early blight, caused by *Alternaria solani* (8, 32). Various reports have shown that uptake and metabolism of several macro- and micro- nutrients are affected by grafting with inter-specific rootstocks (17, 72-75).

In addition to resistance to root-infecting plant pathogens, grafting with diverse rootstocks may also impact other plant-microbe interactions that occur below ground. Plant growth promoting rhizobacteria (PGPR) colonize the roots of plants and induce systemic resistance to insects and pathogens (37, 86). Induced systemic resistance (ISR) mechanisms have been studied in tomato, and several reports indicate that colonization by some PGPR, including *Bacillus* and *Psuedomonas* spp., are mediated by the Jasmonic acid (JA) defense pathway (25, 37, 84, 85). Although studies with the JA pathway indicate that these effects would be graft-transmissible (76), it's not clear if the quantitative level of resistance would be impacted. Clearly, further work is needed in this area.
**Potential pitfalls of grafting:** Although grafting of tomato offers an excellent site-specific soilborne disease management tool, several challenges could arise as a result of the deployment of this technology. One of the primary challenges for successful implementation of grafted plants is proper rootstock selection as many rootstocks confer resistance to one disease, but are susceptible or moderately susceptible to others. For example, the popular tomato rootstock, ‘Maxifort’ is highly resistant to southern blight and carries major resistance genes against races 1 and 2 of *F. oxysporum* f.sp. *lycopersici* (causal agent of fusarium wilt). Although this rootstock performs well against these diseases, it has only intermediate resistance to root-knot nematodes (*Meloidogyne* spp.) and is susceptible to bacterial wilt (*R. solanacearum*). This presents numerous challenges during rootstock selection and requires accurate diagnosis and cropping history.

Even when host resistance is properly deployed, the selection pressure applied to pathogen populations can lead to strains or races that can overcome resistance (50). A classic example of this interaction in plant pathology exists in our current understanding of the nature of resistance to powdery mildew among small grains crops (78). The deployment of a rootstock monoculture may lead to pathogen strains that are able to overcome host resistance. Therefore, future work that explores the rootstock rotation, both spatial and temporal, will be of value. Interestingly, the application of grafting provides a unique opportunity to explore the principles of multilines. Jensen coined the term multiline referring to “…varieties (that) are re-constitutable composites
of phenotypically similar, genetically dissimilar lines” (9, 29). Because grafting provides the opportunity to deploy diverse rootstocks that are grafted onto similar scions, grafting may provide an arena to implement this principle into tomato production.

Another potential challenge to the successful implementation of grafting is that diseases that may be of minor consequence on typical tomato cultivars could emerge as a serious threat to rootstocks. For example, in Italy, grafting has been implemented widely and a recent report suggests that rootstocks are prone to infection by *Colletotrichum coccodes* which causes an unusual root rot disease (51). Other pathogens have been identified on tomato rootstocks like *Phytophthora nicotianae* and *Rhizoctonia solani* (51). Similarly, a bacterial stem rot (caused by *Pectobacterium carotovorum* subsp. *carotovorum* and *P. carotovorum* subsp. *atrosepticum*) was found on grafted eggplants and no symptoms of were observed on nongrafted plants (12). These studies suggest that although grafting with resistant rootstock provides new ways of managing common diseases for growers in the US, caution should be taken to watch for new or unfamiliar diseases when implementing this technology on the farm.
CONCLUSIONS

As opposed to traditional resistance gene deployment in commercial fruiting cultivars, inter-specific rootstocks provide the opportunity to combine major resistance genes with quantitative resistance against soilborne plant pathogens. In recent trials in NC, rootstocks were shown to effectively manage bacterial wilt (67, 71) and southern blight (69), and although resistance has been reported to these diseases in breeding lines, it is not available in commercial cultivars. Grafting provides an accelerated conduit for host resistance deployment and will be a valuable management tool for tomato growers who want to reduce or prevent diseases caused by root-infecting plant pathogens without chemical fumigants.

The utilization of tomato rootstocks may also impact the dynamics of mutualistic plant-microbe interactions belowground, and research with grafted tomatoes could serve as a model for other grafted annual and perennial crops. Furthermore, rootstocks could be identified and/or developed that mediate induced systemic resistance mechanisms with greater efficacy than nongrafted plants. Clearly, further work in this area is needed.

It remains uncertain if tomato grafting will be adopted in the US to the extent that it is practiced worldwide. A number of economic questions still need to be addressed, particularly related to the utilization of grafted transplants in large, open-field conventional farming systems. However, the rapid adoption of high tunnels and
the need for disease management strategies that reduce reliance on chemical
fumigation will make grafting a valuable tool for many growers in the US.
Table 5.1 – Tomato rootstocks evaluated in North Carolina from 2005-2009

<table>
<thead>
<tr>
<th>Rootstocks</th>
<th>TMV</th>
<th>Corky Root</th>
<th>Fusarium Wilt</th>
<th>Verticillium Wilt</th>
<th>Root-knot Nematode</th>
<th>Bacterial Wilt</th>
<th>Southern Blight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beaufort *</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>MR</td>
<td>S</td>
<td>HR</td>
</tr>
<tr>
<td>Maxifort *</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>MR</td>
<td>S</td>
<td>HR</td>
</tr>
<tr>
<td>(Unreleased) *</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>HR</td>
<td>MR</td>
</tr>
<tr>
<td>TMZQ702 **</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>MR</td>
<td>MR</td>
</tr>
<tr>
<td>Dai Honmei ***</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>HR</td>
<td>MR</td>
</tr>
<tr>
<td>RST-04-105 ****</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>MR</td>
<td>MR</td>
</tr>
<tr>
<td>Big Power *****</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>HR</td>
</tr>
<tr>
<td>Robusta *****</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

R=Resistant, HR=Highly Resistant, MR=Moderately Resistant, S=Susceptible
* = De ‘Ruiter Seed Co.  ** = Sakata Seed  *** = Asahi Industries Co.
**** = DP Seeds  ***** = Rijk Zwaan  ****** = Bruinsma Seed Co.
LITERATURE CITED


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APPENDIX A

The following report

Elevation of proteinase inhibitor 2 (PIN 2) expression in tomato as a response to grafting

by

C.L. Rivard, R. Salinas, H.W. Sederoff, and F.J. Louws

was prepared in the format of

HortScience

The American Society for Horticultural Science

Alexandria, VA
ABSTRACT

Although grafting has become relatively common in tomato (*Solanum lycopersicum*) production systems worldwide, little is known about the physiological effect of grafting on specific defense-related mechanisms and how these could be utilized to further develop rootstock breeding programs. The jasmonic acid pathway plays an important role in a number of plant-microbe interactions, and is associated with leaf wounding as a result of insect herbivory. In this study, the systemic expression of *proteinase inhibitor 2* (*PIN 2*) was monitored 1, 2, 4, 8, and 16 days after grafting occurred. *PIN 2* was highly expressed in self-grafted plants as well as those grafted with inter-specific rootstocks, ‘Body’ and ‘Maxifort’, as compared to the nongrafted ones on days 1, 2, 4, and 8 (*P*<0.05), but no effect was seen 16 days after grafting. The plants grafted with ‘Body’ rootstocks had higher *PIN 2* expression than the self-grafted plants on day 4 and plants grafted with ‘Maxifort’ rootstocks had lower levels of *PIN 2* transcripts on day 1 than self-grafted plants. These results suggest that not only does grafting induce the JA pathway, but that rootstock genotype may have a quantitative effect on the systemic expression levels of *PIN 2*. Implications for future research and the role of the wound response in the formation of the graft union are discussed.
INTRODUCTION

Grafting tomato (*Solanum lycopersicum*) seedlings for commercial fruit production purposes is an emerging technology in the US (King et al., 2008; Kubota et al., 2008), and has been utilized worldwide (Lee, 1994; Lee, 2003; Rivero, et al., 2003). In Japan >500 million vegetable transplants are grafted annually (Kubota et al., 2008). Typically, inter-specific hybrids (e.g. *S. lycopersicum* x *S. habrochaites*) are used as rootstocks for tomato, and they provide resistance to multiple soilborne pathogens (King et al., 2008; Rivard and Louws, 2008; Rivard et al., 2010), tolerance of abiotic stress (Black et al., 2003; Rivero et al., 2003), or better fruit yield characteristics as a result of increased plant vigor (Kubota et al., 2008; Lee, 2003). For commercial tomato grafting, the tube grafting method is most common (Lee, 2003). Tomato plants are typically grafted when they are 3-4 weeks old, and the rootstock and scion are held together with a silicone tube/clip while the graft union fuses (Rivard and Louws, 2006).

In addition to resistance to root-infecting plant pathogens, grafting with diverse rootstocks may also impact other plant-microbe interactions that occur below ground. Plant growth promoting rhizobacteria (PGPR) colonize the roots of plants and induce systemic resistance to insects and pathogens (Kloepper et al., 2004; van Loon et al., 1998). Induced systemic resistance (ISR) mechanisms have been studied in tomato, and several reports indicate that colonization by some PGPR, including *Bacillus* and *Psuedomonas* spp., are mediated by the Jasmonic acid (JA) defense pathway (Hase et al., 2008; Kloepper et al., 2004; Valenzuela-Soto et al., 2010; Van der Ent et al., 2009).
Although there are several cases where the JA pathway has been involved in resistance to plant pathogens (Pena-Cortes et al., 2004; Van der Ent et al., 2009; Yu et al., 2009), this defense mechanisms was first described in its relation to insect herbivory (Dicke, 2009; Howe and Jander, 2008; Kessler and Baldwin, 2002), and has historically been associated with wounding (Bostock, 2005; Schilmiller and Howe, 2005; Wasternack et al., 2006). Upon leaf wounding, systemic production of secondary metabolites as well as anti-nutritive compounds occurs. The identity of the systemic signal is still somewhat unclear, but it is graft-transmissible and dependent on JA (Schilmiller and Howe, 2005).

One of the best-studied groups of compounds that are produced in tomato as a result of wounding and subsequent JA-mediated response is the family of protease inhibitors (Ryan, 1990). *Proteinase inhibitor 2 (PIN 2)* encodes one of these compounds and its role in the JA pathway in tomato was described during pioneering studies in this area (Nelson and Ryan 1980). *PIN 2* is regulated via transcription (Farmer et al., 1992) and increases in its expression in tomato have been shown as a result of leaf wounding and exogenous methyl jasmonate applications (Sivasankar et al., 2000), as well as mechanical and chemical induction by glandular trichomes (Peiffer et al., 2009).

Grafting with inter-specific rootstocks could provide a unique opportunity to integrate induced resistance into plant breeding programs by selecting for rootstocks that interact beneficially with PGPR and other microbes below ground. However, before this area of work can be fully explored, the potentially confounding effect of the grafting
procedure must be investigated. The JA pathway has been associated with leaf wounding in numerous reports, but to our knowledge there are no available studies that investigate the effects of stem wounding on tomato. Furthermore, the duration of the activation of the JA pathway as a result of grafting is an important question. If the grafting procedure induces long-term activation of induced resistance mechanisms, then future studies that seek to select rootstocks for increased efficacy of ISR could be compromised by wounding effects related to the grafting procedure.

In order to determine how the grafting procedure affects JA-mediated ISR, quantitative expression of the defense gene \textit{PIN} 2 was monitored over time. Tomato plants were grafted and leaf tissue was sampled 1, 2, 4, 8, and 16 days after grafting. A quantitative RT-PCR protocol was developed and implemented to estimate the systemic expression of \textit{PIN} 2 in scions grafted onto inter-specific rootstocks as well as their own root systems in comparison to nongrafted plants.
MATERIALS AND METHODS

A time course experiment was performed whereby plants were grafted and leaf tissue was collected to determine the quantitative systemic expression of PIN 2. A positive control treatment was also utilized where nongrafted plants were treated with methyl jasmonate (Methyl (1R,2R)-3-Oxo-2-(2Z)-2-pentenyl-cyclopentaneacetate; Fisher Scientific, Pittsburgh, PA). Methyl jasmonate is a chemical elicitor of the JA pathway (Schilmiller and Howe, 2005) and subsequent PIN 2 transcripts (Peiffer et al., 2009; Sivasankar et al., 2000).

**Plant materials.** Greenhouse experiments were performed at the Southeastern Plant Environment Laboratory located at North Carolina State University (Raleigh, NC; http://www.ncsu.edu/phytotron/). ‘Trust’ (Johnny’s Selected Seeds; Winslow, ME) was used as the scion cultivar for grafted treatments and for nongrafted controls. The rootstock cultivars ‘Body’ and ‘Maxifort’ were grafted with ‘Trust’ scions. In addition to the two rootstocks, a self-grafted treatment was included whereby ‘Trust’ scions were grafted back onto their original root systems.

In order to eliminate bias caused by unwanted root and stem wounding, the seeds were sown directly and seedlings were not transplanted throughout the course of the experiment. Plants were grown and subsequently grafted in standard plant propagation trays with 36 (6 cm x 6 cm) cells. Rootstock and scion seeds were sown into the trays in a split-plot randomized complete block design with 6 replications. The
main plots were the sampling points for the time course (1 d, 2 d, 4 d, 8 d, and 16 d), and the grafting treatments were randomized within each main plot. The randomized grafting treatments corresponded to rootstock, non-, and self-grafted treatments. Additional nongrafted 'Trust' plants served as a source of scion material for the rootstocks and also included plants that were to be treated with methyl jasmonate. Greenhouse temperature during the day was 22 degrees C and the nighttime temperature was 18 degrees C. All seedlings were watered twice daily, and were fertilized with a modified Steiner nutrient solution during the morning watering event.

**Application of the treatments and sampling.** Twenty six days after sowing, plants were grafted using the tube grafting technique (Rivard and Louws 2006). Rootstock and scion seedling stems were severed and held together using a silicone clip. Once grafted, the plants were immediately moved into a "healing chamber" where humidity and light conditions were manipulated to promote graft union formation (Rivard and Louws, 2006). During the first 48 hours, plants were held in complete darkness and high relative humidity was maintained (>90%) with a cool-mist water vaporizer. After this initial dark period, low levels of light were allowed into the chamber and incrementally increased during days 3-5 after grafting. Next, humidity levels were reduced gradually and the plants were re-introduced to typical greenhouse conditions 7 days after grafting (Rivard and Louws, 2006).
On the day of grafting, the plants were divided into two groups and placed into separate, but equivalent healing chambers. The first group consisted of the randomized treatments including: nongrafted, self-grafted, and plants grafted with ‘Body’ and ‘Maxifort’ rootstocks. The second group consisted of nongrafted plants that were treated with methyl jasmonate. Methyl jasmonate solution (1 mM) was applied as a foliar spray to the leaves and stems and treated plants were immediately enclosed in several small “bio domes” (Park Seed, Greenwood, SC) for 24 h similar to (Moura and Ryan, 2001). The bio domes were placed inside of a separate healing chamber and after the initial 24 h period, the plants were removed from the bio domes and placed back inside the second healing chamber. This procedure ensured MJ did no cross contaminate to other treatments.

Leaf tissue was sampled 1 day, 2 days, 4 days, 8 days, and 16 days after grafting had occurred. In all cases, sampled plants were removed and discarded. Approximately 100 mg of leaf tissue was removed from the apical meristem of the tomato plants and frozen in liquid N₂ in order to preserve the stability of the RNA. All tissues were stored at -80 degrees C until RNA was isolated for quantification by real time RT-PCR.

**RNA purification and quantification.** RNA was purified with RNeasy Plant Mini-kit (Qiagen, Valencia, CA). Residual DNA was digested on-column using Rnase-Free DNase (Qiagen, Valencia, CA). 100-200 ng purified RNA was annealed to oligo(T) primer
Promega, Madison, WI) and reverse transcribed with M-MLV reverse transcriptase (Promega, Madison, WI). The resulting cDNA was used for quantitative PCR.

PCR primers for quantitative PCR were designed using the PrimerQuest tool (Integrated DNA Technologies, Coralville, IA). Forward primer for PIN 2 (GenBank: KO3291.1) was ACTTGTCCATCTTCTGGATTGCC and the reverse primer was CACATAACACACAACCTTGGATGCCCAC. The primers were blasted to confirm homology and specificity to the gene sequence (http://www.ncbi.nlm.nih.gov/BLAST/).

Primer efficiency was analyzed by comparing the normalized C(T) values of 5 serial dilutions of cDNA and a dissociation curve was plotted to confirm specificity.

Quantitative PCR was performed using a Mx3000P QPCR Detection System (formerly Stratagene, now Agilent Technologies, Santa Clara, CA) and the housekeeping gene, ACTIN2/8 was used to normalize C(T) values (Pandey and Assmann, 2004). SYBR Green PCR mix (Applied Biosystems, Carlsbad, CA) was utilized and each 25 μl reaction mix included 1μl of cDNA template, 3.75μl of each primer (150nM), 12.5μl SYBR Green mix (5mM) and RNase/DNase free water to volume. The PCR program consisted of 15 min at 95º followed by 40 cycles of 95º for 30 s and 59º for 60 s. The real time data were analyzed using the software package included in the Mx3000P QPCR Detection System (Agilent Technologies, Santa Clara, CA). The expression data were determined using the 2-ΔΔC(T) method (Livak and Schmittgen, 2001), with nongrafted plants as the reference group. Expression data were analyzed using the MIXED procedure (SAS 9.1,
SAS Institute, Cary, NC) and an estimate of the means and standard error term was generated for each treatment.
RESULTS

Methyl jasmonate induced high expression levels of PIN 2 as detected by quantitative RT-PCR and the greatest expression of PIN 2 occurred 1 d after treatment (Fig. A.1). Throughout the time course, methyl jasmonate had an elevated but similar effect on the expression of PIN 2 as the grafted treatments (Figs. A.1-4). This indicates that grafting had a similar effect on the systemic expression of PIN 2 as methyl jasmonate, a known chemical elicitor of the JA pathway.

One day after grafting, the self-grafted plants and those grafted onto ‘Body’ rootstock had the highest transcript levels and were similar to each other. Interestingly, plants grafted onto ‘Maxifort’ rootstocks had lower PIN 2 transcripts than the self-grafted plants, but were elevated compared to the nongrafts (Fig. A.1). Two days after grafting, a similar trend was observed and plants grafted onto ‘Body’ had the highest level of PIN 2 transcripts (Fig. A.2). The strong effect of methyl jasmonate on PIN 2 transcript levels was reduced on day two (Fig. A.2) compared to day one (Fig. A.1). Plants grafted with ‘Maxifort’ rootstocks had similar transcript levels to self-grafted plants and those grafted with ‘Body’ two days after grafting (Fig. A.2). On day four, the effect of grafting was somewhat reduced among the self-grafted plants whereas the ones grafted with ‘Body’ and ‘Maxifort’ rootstocks retained elevated expression of PIN 2 (Fig. A.3). Plants grafted with ‘Body’ rootstocks had the highest expression of PIN 2 and plants grafted with ‘Maxifort’ rootstocks had intermediate expression as compared to the self-grafted ones (Fig. A.3). By the eighth day, PIN 2 expression was elevated
(P<0.05; Fig. A.4), but had reduced considerably compared to prior sampling points (Figs. A.1-3).

These results indicate that the mechanical act of grafting increased the level of PIN2 transcripts within the leaf tissue of the scion 1 d post-grafting and continued among all of the grafted treatments until 8 d after grafting (Fig. A.5). Furthermore, PIN2 expression was highest among the grafted treatments 2 d after grafting (Fig. A.5). The plants grafted with ‘Body’ and ‘Maxifort’ rootstocks retained elevated PIN2 expression on day 4 (Fig. A.3) whereas expression in the self-grafted plants was reduced (Fig. A.5). PIN2 transcripts were slightly elevated 8 d after grafting and returned to typical levels 16 d after grafting (Fig. A.5).
CONCLUSIONS

Grafting of herbaceous vegetable crops is a new development in horticultural production and the results of this report indicate that tube grafting of tomato induces the JA defense pathway. Self-grafted plants and those grafted onto inter-specific rootstocks, ‘Body’ and ‘Maxifort’, had elevated expression of PIN 2, a commonly-reported indicator gene of the JA pathway (Peiffer et al., 2009; Ryan, 1990; Sivasankar et al., 2000). Furthermore, grafting with inter-specific rootstocks affected the quantitative level of systemic PIN 2 expression at 1 day and 4 days after grafting. This represents the first report of elevated wound-inducible genes as a result of a stem wounding and/or the grafting procedure in tomato and the time course utilized in this study provides valuable information regarding the effect of grafting on induced resistance mechanisms for this crop.

Previous reports that investigate stem wounding of tomato are rare. However, grafted tomatoes have been used extensively to elucidate the mechanisms of systemic signal cascades related to the JA pathway (Schilmiller and Howe, 2005). In our study, expression of PIN 2 was increased for approximately 8 d. This indicates that the grafting procedure has probably had little confounding effect on previous work with grafted plants. More importantly, it means that the grafting procedure will not inhibit future work to utilize ISR in a production setting with diverse rootstocks. Typically, the grafting procedure requires 7-10 days for complete healing to occur (Fernandez-Garcia et al., 2004; Rivard and Louws, 2006). The results of this study indicate that if waiting
period of 16 days is allowed between the grafting procedure and a potential “inducing” event/treatment, then confounding effects as a result of grafting should not occur.

Based on the time course of PIN 2 expression seen in this study, it may be hypothesized that the duration of the wound response is related to graft union formation. The nongrafted plants that were treated with jasmonic acid had their highest levels of PIN 2 expression 1 d after treatment whereas the grafted plants had their highest expression of PIN 2 on day two. Interestingly, the plants grafted with ‘Body’ rootstocks had higher quantitative expression of PIN 2 on day four than the self-grafted plants and it is not clear what caused this. One factor that can lead to graft incompatibility is poor alignment of the vascular tissues, and this can reduce the likelihood or the speed of graft union formation (Yeoman and Brown, 1976). In the case of the self-grafted plants, the scions were placed back onto their original root systems which could allow for easier reconnection of the vascular tissue.

In a study with pepper, severed stems were submerged in water or solution containing systemin, and unexpectedly, the leaves of plants that were submerged in water produced proteinase inhibitors (Moura and Ryan, 2001). Based on the results of this report, it could be concluded that the unexpected results were due to stem wounding. Our results and others (Peiffer et al., 2009) suggest that further work to elucidate the systemic signal involved in the JA pathway will require a unique approach to circumvent the sensitivity of the JA pathway.
The adoption of tomato grafting may provide a unique opportunity to explore the impact of rootstock genotype on the quantitative level of ISR in above-ground tissues. A recent review highlights the importance of plant roots in providing resistance to pest and pathogen attack (Erb et al., 2009). Our results suggest that although the grafting procedure induces the JA pathway, PIN 2 levels in the grafted plants were similar to those in the nongrafts 8 d after grafting. Future work that seeks to incorporate induced resistance mechanisms into tomato rootstock breeding programs will benefit from this study.
Figure A.1 – Proteinase inhibitor 2 (PIN 2) expression as a result of grafting. Nongrafted and self-grafted ‘Trust’ tomato as well as ‘Trust’ grafted onto ‘Body’ and ‘Maxifort’ rootstocks 1 day (24 h) after grafting. Nongrafts were also treated with Methyl Jasmonate, a chemical inducer of PIN 2. Error bars represent standard error of the mean as determined by proc MIXED (SAS 9.1; SAS Institute, Cary NC).

Figure A.2 – Proteinase inhibitor 2 (PIN 2) expression as a result of grafting. Nongrafted and self-grafted ‘Trust’ tomato as well as ‘Trust’ grafted onto ‘Body’ and ‘Maxifort’ rootstocks 2 days after grafting. Nongrafts were also treated with Methyl Jasmonate, a chemical inducer of PIN 2. Error bars represent standard error of the mean as determined by proc MIXED (SAS 9.1; SAS Institute, Cary NC).
Figure A.3 – Proteinase inhibitor 2 (PIN 2) expression as a result of grafting. Nongrafted and self-grafted ‘Trust’ tomato as well as ‘Trust’ grafted onto ‘Body’ and ‘Maxifort’ rootstocks 4 days after grafting. Nongrafts were also treated with Methyl Jasmonate, a chemical inducer of PIN 2. Error bars represent standard of the mean as determined by proc MIXED (SAS 9.1; SAS Institute, Cary NC).

Figure A.4 – Proteinase inhibitor 2 (PIN 2) expression as a result of grafting. Nongrafted and self-grafted ‘Trust’ tomato as well as ‘Trust’ grafted onto ‘Body’ and ‘Maxifort’ rootstocks 8 days after grafting. Nongrafts were also treated with Methyl Jasmonate, a chemical inducer of PIN 2. Error bars represent standard of the mean as determined by proc MIXED (SAS 9.1; SAS Institute, Cary NC).
Figure A.5 – Timecourse of proteinase inhibitor 2 (PIN 2) expression as a result of grafting. Nongrafted and self-grafted ‘Trust’ tomato as well as ‘Trust’ grafted onto ‘Body’ and ‘Maxifort’ rootstocks were sampled 1, 2, 4, 8, and 16 d post-grafting. Error bars represent standard of the mean as determined by proc MIXED (SAS 9.1; SAS Institute, Cary NC).
LITERATURE CITED


APPENDIX B

The following illustration

A typical timeline for grafted tomato production

by

C.L. Rivard and F.J. Louws

was provided for the publication of

Hartmann and Kester's Plant Propagation:

Prentice Hall
Upper Saddle River, NJ
Available 28 Aug 2010
Figure B.1 – A typical timeline for grafted tomato production.