ABSTRACT

RIVARD, CARY LEE. Grafting Tomato to Manage Soilborne Diseases and Improve Yield in Organic Production Systems. (Under the direction of Frank J. Louws.)

The use of grafted tomato for commercial production has been implemented worldwide, where soilborne disease pressure is high. Grafting has been used to manage *Fusarium*, *Verticillium*, Root-knot nematodes, and bacterial wilt in several Asian, Mediterranean, and northern European countries. However, this technique is relatively unknown in the United States. Recently, direct-marketing avenues for small, sustainable farmers have increased, and consumer-based demand for vine-ripened organic heirloom varieties has made this specialty crop especially important. These cultivars are open-pollinated, and are typically very susceptible to an array of soilborne and foliar diseases. A research program was initiated to investigate the potential of grafting as a major component in an integrated approach to reduce soilborne disease and increase crop productivity for organic heirloom tomato production. Because this research relies heavily on well-developed international techniques and practices, an extension objective was implemented to disseminate information regarding grafting benefits and techniques, and to facilitate local adoption of this technology.

During 2005 and 2006, field trials were implemented to determine the capability of grafting to reduce soilborne disease incidence in heirloom tomato. Bacterial wilt (caused by *Ralstonia solanacearum*) is a devastating soilborne disease in eastern North Carolina. CRA 66 and Hawaii 7996 genotypes were highly effective at reducing bacterial wilt in naturally-infested soils when utilized as a resistant rootstock for heirloom fruit production. No evidence of wilt was seen among resistant rootstock treatments when terminal disease incidence among non-grafted treatments was 75%, and 79% in 2005 and 2006, respectively. Heirloom scion grafted onto rootstock-specific cultivar, ‘Maxifort’, showed no symptoms of fusarium wilt (caused by *Fusarium oxysporum* f.sp. *lycopersici*), and non- and self-grafted controls had 45-50% disease incidence. In the mountain region of NC, verticillium wilt is an especially severe problem for tomato growers as crop rotation is not typically employed. Grafting with ‘Maxifort’ showed high potential as a management tool for this disease based upon increased vigor under continuous and rotational treatments.
Several field trials in 2005 and 2006 investigated the ability of rootstock-specific hybrids to increase crop productivity under organic management practices in a growing environment with little soilborne disease. Grafting with ‘Maxifort’ and ‘Robusta’ did not enhance yields when implemented into a typical on-farm organic production setting. Evaluation of alternative training systems indicated the importance of added vigor by ‘Maxifort’ through enhanced yields under “twin-headed” management in 2005. In 2006, yields were not increased under alternative training methods as compared to standard training system, but grafting with ‘Maxifort’ rootstock showed enhanced crop productivity in both training systems (P=0.005).

Grafting could be a vital component in commercial organic production of heirloom tomato. The unification of heirloom scion with rootstock that confers disease resistance, tolerance to abiotic stressors, and enhanced vigor may be a valuable tool for organic and conventional growers in the United States.
GRAFTING TOMATO TO MANAGE SOILBORNE DISEASES
AND IMPROVE YIELD IN ORGANIC PRODUCTION SYSTEMS

By

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DEDICATION

To my loving wife, Tia, and son, Samuel; tomatoes get boring, beer goes flat, but my family continues to enlighten my life more and more, each and everyday.
BIOGRAPHY

Cary Rivard was born in Kansas City, MO on June 26, 1981. He grew up in a family-owned and operated retail production greenhouse, which was established in 1991. It was here that he gained his first experiences with plant propagation and production. He graduated from Lee’s Summit High School in 1999, and earned a B.S. degree from Truman State University (Kirksville, MO) in 2005. 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. Furthermore, he 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, and obtained a graduate research assistant position in the Department of Plant Pathology in the summer of 2005.
ACKNOWLEDGEMENTS

I would like to acknowledge everyone in my life that has had an affect on me, 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 maintained without the excellent help of Jim Driver, Rob Welker, Mike Carnes, Amy Keeter, Amanda Guichard, and Tray Bridgers. I appreciate the kind words, teaching, and support that I receive from the students, faculty, and staff in the Department of Plant Pathology at NCSU. I would also like to thank my gracious on-farm collaborators including: Richard Thomas, Randall Patterson, Alex and Betsy Hitt, Ken Dawson, Greg Hoyt (NCSU), Bryan Green (CEFS), and everyone at the NCSU Phytotron. Thanks to Dr. Jay Scott (IFAS) for his expertise and excellent help in identifying and providing resistant genotypes. I thank my committee members for all of their thoughts and ideas that have added to the development of this research program at NCSU, and hope that as I continue into my PhD program, we can foster its further development.

I would like to thank my friends and family for putting up with me for all of these years. My parents: for giving me the encouragement and opportunity to forge my own. My brother: who actually suggested that I completely disassemble a 1998 Toyota engine and put it back together again. Jule and Betty See: for helping a 13 year-old kid open up a tomato stand on the neighborhood corner. All my friends along the way including, but not limited to: Chris for his words of encouragement, Fred for his words of wisdom, and Rutter for his unending stream of words. And of course to my wife and son; whose continued love and support have been inspirational throughout this journey we call grad school.
# GRAFTING TOMATO TO MANAGE SOILBORNE DISEASES AND IMPROVE YIELD IN ORGANIC PRODUCTION SYSTEMS

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CHAPTER ONE – REVIEW OF THE LITERATURE

SECTION ONE – INTRODUCTION

Bacterial wilt, caused by *Ralstonia solanacearum* is a problematic soilborne disease in many southeastern crops including: tomato, potato, tobacco, eggplant and a few ornamental plants, such as geranium. This bacterium causes severe losses worldwide due to its wide geographic distribution and an unusually broad host range, including more than 50 plant families (Kelman 1998). It is recognized as a soil inhabitant and is able to persevere in the soil environment through long crop rotations. Furthermore, it can easily move into non-infested areas through water, soil, infected plant material, and by mechanical means.

The cultivation of grafted vegetable plants began in Korea and Japan at the end of the 1920’s when watermelon plants were grafted onto squash rootstock (Rivero, Ruiz, and Romero 2003). Since then, grafting has been adopted throughout Asia and Europe. Currently, 81% and 54% of vegetable cultivation in Korea and Japan, respectively, uses grafting (Lee 2003). This cultural technique is mainly utilized in intensive cropping systems like greenhouse and tunnel production. Grafting is especially popular for tomato, eggplant and cucurbit production in Asia, and in 1998, 540 million transplants were grafted in Korea and 750 million in Japan (Lee, Bang, and Ham 1998). This technique has been adopted in the Mediterranean region as well, where the use of grafting has been proposed as a major component of an integrated management strategy for managing soilborne disease and increasing crop productivity. Grafted tomato has increased in Spain from less than one million plants in 1999-2000 to over 45 million plants in 2003-2004 (Besri 2005). Grafted tomato is also cultivated in France, and Italy,
and over 20 million tomato plants were grafted in Morocco in 2004 as a way to reduce soilborne disease and increase the length of the harvest season (Besri 2005).

Grafting could be a major component in American agricultural practices due to the combined benefits associated with soilborne disease resistance and enhanced crop productivity through highly vigorous rootstock genotypes. Furthermore, this technology could be instrumental for organic and sustainable production of heirloom tomato.

Agriculture in America is changing. Everyday, organic practices are more widely encouraged in US markets. Furthermore, the reappearance of the farmer’s market as a viable marketing tool, has made small-scale sustainable farming ever more popular. Heirloom tomatoes are an extremely important component of these markets as local chefs and food connoisseurs have pronounced these varieties as having superior flavor characteristics. Furthermore, these varieties increase farm-gate income due to their high consumer-based demand. However, heirloom tomatoes are extremely susceptible to an array of soilborne and foliar diseases. Bacterial wilt and other soilborne diseases consistently plague heirloom tomato growers in the southeastern United States. This problem will continue to grow as Early Blight, caused by Alternaria solani, has forced many organic tomato production systems to move inside of greenhouses and other tunnel systems. This transition may ultimately lead to increased severity of bacterial wilt and other soilborne diseases as the length of crop rotations are decreased within these growing environments. The use of resistant rootstock grafted onto heirloom scion may be able to unite the soilborne disease resistance characteristics needed for an integrated production approach with the high quality fruit demanded by local markets.
SECTION TWO – SOUTHERN BACTERIAL WILT OF TOMATO

Southern bacterial wilt of tomato (caused by *Ralstonia solanacearum*) is a devastating disease throughout the southeastern growing region of the United States. In eastern North Carolina, infested fields have been totally abandoned as a result of consistent pressure from *Ralstonia solanacearum* in tobacco, and tomato production. Much of North Carolina’s tomato production has moved to the western regions, where bacterial wilt pressure is less severe. However, through nationwide movement of transplants and reoccurring flooding events, this region is beginning to see isolated appearances of this disease (Rivard, unpublished). Crop rotation with a non-susceptible host may provide some control, but this measure is difficult because of the wide host range of the pathogen and the considerable value of local agricultural lands. Methyl bromide has been historically used in North Carolina as a broad-spectrum biocide that eliminates pressure from various soilborne pathogens, nematodes and weeds. However, the use of methyl bromide has led to the deterioration of the ozone layer, and has been recently phased out for use under normal agricultural conditions. Furthermore, methyl bromide is not effective due to high re-colonization rates and dissemination of *R. solanacearum* through irrigation and floodwaters (Driver and Louws 2002; McCarter 1991).
*Ralstonia solanacearum* – Biology and Epidemiology of the Pathogen

*Ralstonia solanacearum* is a gram-negative rod bacterium, 0.5-0.7 X 1.5 – 2.0 \( \mu \)m, and is motile by one to four polar flagella (Driver and Louws 2002; McCarter 1991). It inhabits the soil of tropical and sub-tropical environments, and is metabolically versatile, surviving and thriving in such diverse habitats as water, soil, and latently infected plants (Hayward 1991). The bacterium invades plant roots from the soil through wounds or natural openings, and colonizes the xylem tissue, preventing water movement into the upper portions of the plant. Symptoms of the disease first appear as flaccidity in one or more of the youngest leaves. Under favorable conditions, total collapse of the plant may occur in 2-3 days. Leaf tissue remains green throughout this process, and the only apparent symptom of the pathogen in the field besides leaf flaccidity is browning of the xylem tissue. In some cases, adventitious roots may develop under less than optimum conditions for disease development or with the deployment of moderately resistant cultivars (McCarter 1991). Bacterial wilt can be easily distinguished from fungal wilt diseases due to the milky-white exudation of bacterial cells from infected stem tissue.

*Ralstonia solanacearum* was first described as a plant pathogen by Erwin F. Smith in 1896, and although the causal agent had not been described prior to Smith’s work, farmers were well aware of its destruction as it devastated economically important crops worldwide. Smith continued to work with this plant pathogen, and soon found that it was capable of rapidly losing virulence in culture (Kelman 1998). *Ralstonia solanacearum* enters plant roots from the soil through wounds or natural openings. Transplanting, insects, nematodes, or mechanical wounding by agricultural equipment can cause these infection courts. Furthermore, *R. solanacearum* may infect the
undisturbed root system of a susceptible host through microscopic wounds caused by the emergence of lateral roots (McCarter 1991). Colonization by the bacteria within the xylem prevents water movement into the upper portions of the plant tissue, and is not known to be associated with toxin production (Kelman 1998). However, \textit{R. solanacearum} is known to produce extracellular polysaccharides, which accumulate within and clog the vascular tissue, ultimately leading to total wilt of the plant (Husain and Kelman 1958).

\textit{Ralstonia solanacearum} attacks over 200 species of cultivated plants and weeds in 50 plant families (Kelman 1998). Among this broad range of hosts, the most economically important are solanaceous: tobacco, tomato, potato, and eggplant. Banana is also a host of considerable importance (McCarter 1991). The difficulty in managing this disease is not only due to its broad host range, but also because the organism survives in the soil for extended periods in the absence of host plants. It is released into the soil from the roots of infected plants and from decaying infected host material. Within the soil, the survival periods may vary based upon the strain type and the characteristics of the soil environment. Typically, well-drained soils with good water retention characteristics are conducive to survival of the pathogen (McCarter 1991). Movement of water both under the soil surface as well as aboveground can lead to long-distance dissemination of pathogen inoculum (Momol et al. 2005).

Motility of this bacterium important for its dissemination and recent findings have shown that chemotaxis plays a role during the host-pathogen interaction. Qualitative and quantitative chemotaxis assays revealed that this bacterium is specifically attracted to diverse amino acids and organic acids, and especially to root exudates from the tomato
host plant (Yao and Allen 2006). The ability of *Ralstonia* to seek out its host within the soil environment is an attribute that leads to the aggressiveness of this disease.

*Ralstonia solanacearum* comprises a complex species and maintains considerable diversity. The world population has been separated into four biovars (Hayward 1964) and three races based upon host range (Buddenhagen and Kelman 1964). In the US, biovar 1, race 1, is of greatest importance, as it occurs endemically in the southeastern regions, and the disease it causes is commonly referred to as Southern bacterial wilt (McCarter 1991).
Methods for Control

Although bacterial wilt continues to plague many growers worldwide, some success has been found with the use of cultural management practices such as crop rotation. Non-host plants can be rotated into an agricultural system in order to reduce the population size of a given soilborne pathogen. This practice can lead to a decrease in primary inoculum from one year to the next. In Nepal, a susceptible tomato cultivar was rotated with corn (*Zea mays*), lady’s finger (*Abelomoschus esculentum*), cowpea (*Vigna unguiculata*), and a partially resistant tomato cultivar (Adhikari and Basnyat 1998). Wilt severity was significantly reduced, and the onset of bacterial wilt was delayed by 1-3 weeks for the susceptible and partially resistant cultivars. Furthermore, the partially resistant cultivar had an even further decrease in wilt severity and increase in yield as these two management strategies appeared to be working synergistically (Adhikari and Basnyat 1998). The integrated use of partial resistance and crop rotation showed additive affects in managing this disease in tobacco as well (Melton and Powell 1991). In potato, one study found similar results. A two-year rotation was able to reduce bacterial wilt disease incidence in heavily infested fields, and a one-year rotation could reduce wilt incidence in mildly infested fields (Lemaga et al. 2001). Both of these trends indicate the importance of reducing the primary inoculum load in infested soils through crop rotation.

Although rotation may be able to reduce bacterial populations in the soil, good sanitation methods and monitoring for weeds can be very important for managing bacterial wilt. *Ralstonia solanacearum* is capable of surviving in the rhizosphere and the roots of symptomless weed species (Granada and Sequeira 1981). Interestingly, solanaceous weeds along a riverbank contributed the primary inoculum source to the
irrigation water, and only after a proper weed eradication program was initiated, did the disease become manageable (Umaerus 1992). Similarly, proper sanitation can be very important to decrease bacterial wilt in the field. *Ralstonia solanacearum* has been shown to survive among agricultural equipment such as packing crates and other wood-based products (di Bisceglie et al. 2005). The bacterium may also be spread through irrigation water, and treatments may reduce the incidence of bacterial wilt in the field (Momol et al. 2005). Several studies have alluded to the importance of pH for cultural management of bacterial wilt. Bacterial wilt does not occur in the basic, calcareous soils of Florida (Momol et al. 2005). Similarly, the use of urea and calcium oxide soil amendments was far more successful at higher pH ranges (Michel et al. 1997).

The use of chemical fumigants to control bacterial wilt in field production has been relatively unsuccessful. Methyl bromide is a broad-spectrum biocide that has historically been used to control soilborne disease in raised bed, plasticulture systems. This fumigant had no significant effect upon the incidence of bacterial wilt in several North Carolina field trials. Similarly, Telone-C35 and Chloropicrin showed no efficacy in controlling this disease (Driver and Louws 2002).

The most commonly cited commercially-available chemical formulation for control of bacterial wilt is acibenzolar-S-methyl, commonly known as Actigard. Actigard is a chemical elicitor that induces the systemic acquired resistance (SAR) defense pathway. This defense pathway is known to reduce the effects of many foliar and soilborne diseases (Collins et al. 2006; Louws et al. 2001; Obradovic et al. 2004). It has been effective against bacterial wilt when inoculum densities are low (Anith et al. 2004). In another study, the use of Actigard was not shown to be effective in severely
infested fields where a susceptible cultivar was used. However, when a moderately
resistant cultivar was deployed, the integrated use of both of these management tactics
proved to be very successful (Pradhanang et al. 2005).

Other more novel chemical approaches for managing bacterial wilt have recently
been developed. A soil amendment consisting of calcium oxide and urea was applied in
field studies in Taiwan, and was shown to significantly reduce bacterial wilt in tomato
(Michel et al. 1997). In contrast, these results were supported further when it was
incorporated into various soil types in laboratory microcosm experiments (Michel and
Mew 1998). Silicon-based soil amendments were effective at reducing bacterial wilt
disease incidence as expressed by the area under disease progress curve for susceptible
and partially resistant cultivars (Dannon and Wydra 2004). A soil drench consisting of
phosphorous acid (H₃PO₃) was successful at eliminating the incidence of bacterial wilt in
ornamental geranium (Norman et al. 2006). Phosphoric acid may protect plants from
infection by acting as a bacteriostatic compound in the soil, as it was found to inhibit
growth of _R. solanacearum_ in vitro (Norman et al. 2006). Two reduced risk fumigants
have shown moderate efficacy against _R. solanacearum_ at reducing wilt incidence in the
field. The use of thymol and palmarosa oil as biofumigants resulted in 33.1% and 48.1%
disease incidence respectively, whereas the control plots contained 92.5% wilt. In the
following year, disease incidence in untreated plots was 62.5%, and thymol treatments
showed a 12% disease incidence rating (Ji et al. 2005).

Because conventional methods of control have shown low efficacy, biological
control is a promising alternative for the control of bacterial wilt in tomato. One strategy
for biological control involves the use of plant growth promoting rhizobacteria (PGPR).
These bacteria colonize the rhizosphere of the crop plant and protect it from invasion by soilborne pathogens, or activate endogenous plant defense pathways (Kloepper and Schroth 1979). In China, four field trials were conducted to evaluate three PGPR strains, *Serratia* spp. J2, fluorescent pseudomonad J3 and *Bacillus* spp. BB11. All of these strains were shown to be effective at reducing wilt incidence in the field and yields were significantly higher than in untreated control treatments. Furthermore, populations of these PGPRs in one-year-old formulations were equal to freshly prepared PGPR formulations, indicating the potential for future potential commercial formulation (Guo et al. 2004). Research with streptomycetes showed that these rhizosphere-colonizing bacteria were able to inhibit *R. solanacearum in vitro*. Further experiments showed that this PGPR was able to significantly reduce bacterial wilt incidence in infested soils compared to untreated control treatments (Gava et al. 2002).

Other methods of biological control like the incorporation of the arbuscular mycorrhizal fungus, *Glomus versiforme*, have shown to reduce *R. solanacearum* populations in the rhizosphere, root surfaces and in the xylem. Furthermore colonization by *G. versiforme* induced the production of soluble phenols in the plant tissue both locally and systemically (Zhu and Yao 2004). *Pythium oligandrum* (PO) is an effective biological control agent on a wide range of fungi due to the elicitor activity of the cell wall proteins (CWP) within this fungus to induce defense responses in plants and its ability to act as a mycoparasite. When mycelial homogenate was applied to tomato roots, PO-treated plants showed enhanced resistance to *R. solanacearum* and a reduction in severity of wilt symptoms. Furthermore, the level of ethylene in PO- and CWP-treated plants was transiently elevated within 8 hours after treatment, followed by high
expression of three basic ethylene-inducible defense-related genes (Hase et al. 2006). Through induction of endogenous plant defense pathways, biological control may be an effective component for management of bacterial wilt in tomato.

The use of host resistance to control bacterial wilt in the field has been very difficult due to the nature of the pathogen and host resistance in tomato. In tobacco, the presence of a major resistance gene has led to the development of genotypes that are capable of carrying out the hypersensitive response, characteristic of a gene-for-gene interaction (Robertson et al. 2004). However, a similar major resistance gene in tomato has not been found to date, and the complexity of this pathosystem continues to elude plant breeders.

Development of horticulturally acceptable tomato varieties with resistance to bacterial wilt in the field has been a significant challenge as evidenced by the lack of such varieties in many growing regions where the disease is a major constraint. Resistance is complex, being controlled quantitatively and strongly influenced by environmental conditions such as soil temperature, pH, and moisture (Scott, Wang, and Hanson 2005). Large variations in wilt severity were observed for several genotypes in warm and cool growing conditions (Prior et al. 1996). The complex diversity of pathogenic Ralstonia strains has led to the development of resistant lines which are effective in some growing regions and not effective in others (Scott 1996). Historically, the pathogens of tomato have been in the race 1 group, and could be derived from biovar 1, 3, or 4. However, a potato strain has recently been shown to infect tomato, and this is causing problems in several growing regions (Elphinstone 1994). Tomato genotypes with bacterial wilt resistance originated from wild tomato, particularly L. esculentum var. cerasiforme and L.
*pimpinellifolium.* It is generally accepted that resistance from various sources is controlled multigenically and usually the resistance is not complete and breaks down to some extent when conditions favor the pathogen (Danesh et al. 1994; Hartman and Elphinstone 1994). However, because genetic evidence supports the presence of single-gene resistance and oligogenic resistance, the exact nature of bacterial wilt resistance inheritance is a topic of debate among plant breeders (Scott, Wang, and Hanson 2005).

The difficulty in breeding large-fruited, horticulturally-acceptable varieties has been well documented (Opena et al. 1990; Walter 1967; Wang, Hanson, and Barnes 1998) and there is a lack of commercially-acceptable resistant varieties currently available to growers. ‘Neptune’ is a large-fruited line developed and released by JW Scott (IFAS), and although it is moderately resistant, it does not contain the complete resistance available in wild tomato genotypes (Scott et al. 1995). Recently, a worldwide study was carried out evaluating 31 genotypes from at least 14 resistance sources in 11 countries (Wang, Hanson, and Barnes 1998). Of all genotypes through the study, Hawaii 7996 had the highest and most consistent survival percentages with a mean of 97% and a range of 85-100%. In fact, three Hawaiian lines (7996, 7997, 7998), all scored very well in regards to host resistance. ‘Neptune’ is derived from Hawaii 7997, but its resistance is not as high as the source of resistance, indicating the involvement of oligogenic inheritance (Scott, Wang, and Hanson 2005). Furthermore, backcrosses between the resistant Hawaii 7998 and susceptible ‘Walter’ genotype showed a 1:1 ratio, as expected with a single dominant gene. However, segregation in the F2 deviated significantly from the 3:1 ratio, suggesting control by a more than a single dominant gene (Scott, Wang, and Hanson 2005). In contrast, although Hawaii 7998 has been shown to confer resistance, it
consistently shows less resistance than 7997 and 7996 (Scott, Somodi, and Jones 1993). All of these results suggest that one or a few major resistance genes together with several minor genes condition resistance to bacterial wilt in tomato (Scott, Wang, and Hanson 2005).

Although the integration of resistance genes into modern tomato cultivars has been difficult, the use of grafted transplants has historically been very effective for managing bacterial wilt in the field worldwide. CRA 66 has been identified as a resistant rootstock variety for grafted tomato production in India and Germany (Grimault and Prior 1994; Tikoo, Mathai, and Kishan 1979). Grafting has been a practical and effective management tool for bacterial wilt worldwide, and will be discussed further in the following section.

Early investigations of the physiological mechanisms involved with resistance to bacterial wilt in tomato suggested that resistant host genotypes physically limit the movement of the bacteria from the soil environment into the collar and mid-stem portions of the plant (Grimault et al. 1994). Susceptible scion grafted onto resistant rootstock wilted under inoculation of the scion-derived stem and adventitious roots (Obrero, Aragaki, and Trujillo 1971). Reciprocal grafting experiments showed that resistant scions grafted onto susceptible rootstock resulted in wilt and bacterial colonization by *R. solanacearum*. Furthermore, examination of resistant cultivar ‘Caraibo’ indicated that tylose formation may be involved with the physical limitation of *Ralstonia* through the vascular tissue (Grimault et al. 1994). Further work examined the resistance level of 13 tomato genotypes from varying backgrounds. Among the genotypes tested, CRA 66 (*L. esculentum var. cerasiforme*) and Hawaii 7996 (*L. pimpinellifolium*) showed the fewest
number of symptomatic plants, and a distinct correlation between the bacterial colonization index at the mid-stem and collars of host plants and wilt severity was observed (Prior et al. 1996). More recently, histological observation of bacterial movement through resistant and susceptible varieties suggests that bacteria are able to colonize primary xylem tissue, but are not capable of moving into secondary xylem tissue in the stem of a resistant genotype (Nakaho, Hibino, and Miyagawa 2000). In a more comprehensive review of several genotypes, bacterial colonization within Hawaii 7996 was limited to the protoxylem, one component of the primary xylem while other resistant cultivars limited colonization to all parts of the primary xylem. In all resistant cultivars, however, colonization of the secondary xylem did not occur whereas in susceptible interactions, it did. In a subsequent screen of several lines for rootstock use, latent infections occurred when a resistant cultivar was employed as a rootstock (Nakaho, Hibino, and Miyagawa 2000). In this case, colonization 1 cm above the graft union occurred in 38.7% of inoculated plants, although these infections showed no symptoms of wilt. In contrast, the self-grafted susceptible scion cultivar showed 100% wilt 14 days after inoculation. These experiments suggest that susceptible scions on resistant rootstocks often become infected from bacteria in the rootstocks, but multiplication in scions may be below the threshold for the onset of wilt (Nakaho et al. 2004). Further work is needed to determine the physiological and molecular aspects of host resistance in this pathosystem.
SECTION THREE – VEGETABLE GRAFTING AS AN IPM TOOL

Although the specific historical origin of grafting is unknown, the Chinese were grafting plants by 1560 BC. Both Aristotle (384-322 BC) and Theophrastus (371-287 BC) wrote about grafting, and even suggested the idea of graft compatibility that still plagues researchers today. During the reign of the Roman Empire, grafting came into common horticultural use. Paul the Apostle discussed grafting “good” olives onto “wild” olives in Romans 11:16-24 (Ombrello 2006). Grafting of perennial horticultural crops such as fruits and ornamentals is a prevalent practice in plant propagation. In these crops, the graft is made during the dormant season, and healing occurs as the plant begins to grow in the spring. The worldwide use of soft-tissue grafting, however, is becoming evermore popular in annual vegetable production systems, as environmental sustainability as well as intensive production has increased in many countries.

A number of crops can be grafted. However, because of the added expense, it is typically associated with high-value horticultural crops such as cucurbits and members of the Solanaceae family. In many Mediterranean countries, grafting is being used to control root-knot nematodes, bacterial wilt, and other soilborne pathogens, as an alternative to methyl bromide applications (Ioannou 2001).

There are a variety of methods for grafting vegetable crops. Cleft grafting occurs when a V-shape is cut into the rootstock and a complementing wedge-shaped scion is inserted. The graft is then held with a small clip until healing occurs (Oda 1999). Tongue grafting involves notching opposing sides of the stems of the rootstock and scion, and then using a clip to hold the stems together while they fuse. Once the graft has healed, the original scion is then cut off of the desired rootstock and the unused rootstock
is detached from the scion (Lee 1994). Micrografting is a new technique that has been recently integrated into micropropagation production for hybrid tomato. This method uses micropropagated scion shoots that grafted onto 3 week-old rootstock seedlings (Grigoriadis, Nianiou-Obeidat, and Tsaftaris 2005).

The most common commercial technique for grafting tomato is tube grafting. Tube grafting takes place when the scion and rootstock are severed as seedlings and reattached with a small, silicon tube or clip (Oda 1995). This technique has been highly effective as it can be carried out when plants are very small, thereby eliminating the need for large healing chambers while increasing throughput. Tube grafting has been adopted as the primary method for vegetable grafting on the farm as it can be easily carried out with small healing chambers with typical success rates ranging from 85 to 90 percent (Oda 1995).

During the healing process, meristematic tissue must develop into vascular tissue that will reconnect the scion and rootstock. An experiment that used a non-destructive method to assess the development of hydraulic connections in the graft union of tomato showed that the graft union is completely functional 6-8 days after grafting (Turquois and Malone 1996). Furthermore, measurements carried out above and below the graft union gave similar conductance ($L_O$) values, suggesting that the graft union cannot be considered a barrier to water flow (Fernandez-Garcia et al. 2002). Both of these studies were in agreement, pointing out that the graft union acts as a continuous unit in respect to water movement. In a more recent study, the developmental timeline for graft union formation in tomato is better described (Fernandez-Garcia, Carvajal, and Olmos 2004). The early stage begins within 4 days, and is characterized by the death of cell layers at
the graft interface as a result of wounding. Generation of parenchymatous callus tissue fills the gap between the rootstock and the scion, and living cells from the surface quickly begin to grow in size and divide. Differentiation of callus parenchyma into new cambial tissue occurs, and the subsequent union of the newly formed vascular strand with the original vascular bundle in both the rootstock and the scion begins between days 4 and 8 days. This differentiation and union formation process is fully developed after 15 days (Fernandez-Garcia, Carvajal, and Olmos 2004). These results generally agree with previous studies, although the specific timing may be altered slightly by the environmental conditions surrounding the grafting process. Tissue analysis showed the importance of peroxidase activity during the graft union formation and development, and it has been suggested that this enzyme plays a role in lignification of xylem vessels (Fernandez-Garcia, Carvajal, and Olmos 2004).
Resistance to Biotic Stressors

Grafted vegetable crops have been used extensively in greenhouse and tunnel production as a way to decrease reliance on chemical fumigants. Because these operations typically do not use crop rotation, high levels of soilborne pathogen inoculum can lead to significant disease incidence and ultimately, crop failure. Even when crop rotations are available, the long intervals required between similar crops result in an economic loss to the grower. In field-grown conventional “non-grafted” chemical fumigants are utilized to decrease soilborne disease levels. The implementation of grafting can reduce disease in operations where fumigants are either unwanted or unavailable (Oda 1999).

The first grafts in the early 20th century were made in order to diminish soilborne pathogens such as *Fusarium oxysporum* on watermelons (Rivero, Ruiz, and Romero 2003). However, research has shown that this technique can be effective against a variety of fungal, bacterial, viral, and nematode diseases. Furthermore, many studies have shown that the use of this technology can be equally as effective in managing soilborne disease as methyl bromide—a highly toxic fumigant that has been widely used until recently (Besri 2001; Bletsos 2005; Giannakou and Karpouzas 2003; Pavlou, Vakalounakis, and Ligoxigakis 2002).

Grafting can be an excellent management technique for soilborne fungal pathogens. Grafting has been utilized to eliminate Verticillium and Fusarium wilt in melon and cucumber production in Japan, Korea, and Greece (Bletsos 2005; Ioannou 2001; Oda 1999). In tomato, grafting with resistant rootstock has been effective against *Verticillium dahliae* as well (Ioannou 2001; Tsror and Nachmias 1995). Interestingly, in
potato, stem-dipping experiments with reciprocal grafts showed that tolerance was independent of the root genotype when a resistant genotype was employed as either the rootstock or the scion (Tsror and Nachmias 1995). Grafted tomatoes in New Zealand were able to reduce levels of corky root rot, caused by *Pyrenochaeta lycopersici*, leading to a highly developed root system and ultimately increased nutrient uptake (Bradley 1968). Resistance to this pathogen has been shown in other studies as well (Ioannou 2001). Grafting onto resistant rootstocks showed enhanced yield under infestation of root and stem rot of cucumber, caused by *Fusarium oxysporum* f. sp. *radicis-cucumerinum*, an emerging catastrophic disease in greenhouse cucumber production (Pavlou, Vakalounakis, and Ligoxigakis 2002). Experiments in soils infested with *Phytophthora cryptogea* showed that this technology could be used to manage root rot in greenhouse tomato (Upstone 1968). Grafting can be an excellent management technique for soilborne fungal pathogens.

In Morocco, grafting is used commercially to control root-knot nematodes and other soilborne diseases in over 2000 ha of greenhouse tomato, melon and watermelon (Abdelhaq 2004; Besri 2001). Grafting with resistant rootstock has been successful for cucumbers against root-knot nematodes (*Meloidogyne* spp.) in Greece (Giannakou and Karpouzas 2003). Grafting onto resistant tomato rootstock for greenhouse production has also been adopted in eggplant production in this region. This technique is highly effective for managing root-knot nematodes, and provides equivalent control as compared to fumigants in winter production (Ioannou 2001). Similarly, eggplant rootstocks may provide resistance to root-knot nematodes for eggplant production (Rahman et al. 2002). In summer crops, the resistance of the *Mi* gene in tomato may
break down as a result of high soil temperatures (Ioannou 2001). This phenomenon was first observed early in the investigations regarding nematode resistance (Dropkin 1969). In one study, the use of Mi gene-resistant rootstock has shown increased yields under high nematode pressure even though galling indexes remain high (Lopez-Perez et al. 2006). The Mi gene may confer tolerance rather than complete resistance to root-knot nematodes. In either case, the implementation of the Mi gene in rootstocks can be used to increase yields in infested soils.

While there are very few cited cases, grafting may be used to manage viral diseases as well. Tomato yellow leaf curl virus (ToYLCV) is transmitted by Bemisia tabaci, the sweet potato whitefly, and the use of highly vigorous rootstock has shown to be effective for managing this disease (Rivero, Ruiz, and Romero 2003).

Although grafting has become increasingly important for all soilborne diseases, it is fundamental in reducing damage caused by bacterial wilt (Ralstonia solanacearum) of tomato. This particular disease requires long rotation times in order to successfully eliminate primary inoculum. Furthermore, breeders continue to struggle since the genetic traits for resistance are tightly linked with genetic traits associated with poor fruit quality. Thus far, attempts to uncouple these traits have been relatively unsuccessful. Grafting has been essential in Asian horticultural production for eliminating bacterial wilt incidence in solanaceous crops (Oda 1999). It has also been used in tropical environments, like Brunei, where bacterial wilt incidence is so high that tomatoes cannot be planted unless the soil is sterilized or resistant rootstocks are implemented (Peregrine and Binahmad 1982). In India, CRA 66 rootstocks reduced bacterial wilt incidence in tomato, and plant survivability rates at 1st harvest increased from 54.5% in the control to
100% (Tikoo, Mathai, and Kishan 1979). By the end of the season, none of the control plants had survived while 100% of the grafted plants continued to produce. Furthermore, the yield of the tomatoes with resistant rootstocks was four times that of the susceptible lines. CRA 66 was used against bacterial wilt in Germany and similar results were found (Grimault and Prior 1994). Several Hawaiian lines (Hawaii 7996-7998) have been identified as suitable candidates for resistance to bacterial wilt (Oda 1999; Tresky and Walz 1997). The use of wild eggplant genotypes for rootstock in tomato production has also been well-documented (Matsuzoe, Okubo, and Fujieda 1993). Wild eggplant rootstocks are highly resistant to bacterial wilt as well as root-knot nematodes, but are typically not as vigorous as tomato genotypes. Because the plants may be in a state of water-deficiency, fruit quality is higher with the use of these eggplant genotypes (Matsuzoe et al. 1996).

Grafting has shown to be an effective tool in an integrated pest management system as a broad-spectrum management tool in a number of vegetable crops. As the phase-out of methyl bromide continues, this technique will prove to be an instrumental alternative to this fumigant. Similarly, the combined use of grafting with other techniques such as soil solarization and other chemical alternatives can have a synergistic affect in decreasing soilborne disease and improving crop productivity (Bletsos 2005; Giannakou and Karpouzas 2003; Ioannou 2001).
Tolerance of Abiotic Stressors

Grafting has been highly effective at overcoming abiotic stressors such as salinity, temperature extremes, and excessive soil moisture. As a result of over-fertilization and desertification, over 1/3 of all the irrigated land in the world is affected by salinity, and this technology could be instrumental in decreasing yield losses (Rivero, Ruiz, and Romero 2003). Grafting has also been utilized in order to reduce the effects of flooding in areas where a wet season may occur (Black et al. 2003).

Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. A number of strategies have been developed to overcome the deleterious effect of salinity including transgene deployment, use of QTL markers, cultural techniques, and land-use management (Cuartero et al. 2006). Grafting tomato with tolerant rootstock genotypes has been highly effective at producing a saline-tolerant plant, as indicated by plant growth (Fernandez-Garcia et al. 2004). At high levels of NaCl, the use of ‘Radja’, ‘Pera’ and hybrid ‘Volgogradskij’ x ‘Pera’ as rootstocks was able to increase yields up to 80% as compared to non-grafted and self-grafted controls. Furthermore, leaf concentrations of Na+ and Cl- indicated that these rootstocks prevented the translocation of these deleterious ions into the shoot (Estan et al. 2005). Interestingly, tobacco scion grafted onto salt-tolerant tomato rootstock showed similar results (Ruiz et al. 2005). Grafted watermelons with saline-tolerant rootstocks showed yield increases up to 81% under greenhouse production in the Mediterranean (Colla et al. 2006). The use of salt-tolerant rootstock may be an important management tool for vegetable production until better land-use methods have reduced the severity of this problem in arid and semi-arid conditions.
Grafting has also been effective at reducing damage caused by soil temperature extremes. Because soil tends to heat and cool much more slowly than the air temperature, rootstock tolerant to thermal stress can affect crop productivity more than aboveground structures (Rivero, Ruiz, and Romero 2003). Many of the most economically important crops, such as tomato, squash, cucumber, and watermelon are highly sensitive to cold temperatures throughout vegetative development and reproduction (Jackman et al. 1988). During winter cultivation, the rootstocks ‘Renova’ and ‘Esvier’ significantly promoted vegetative growth of the scion in cucumber production (Zijlstra, Groot, and Jansen 1994). Furthermore, these effects were not seen during summer production. It has been proposed that the ability of the rootstock to withstand colder temperatures is based upon lipid differences in the membranes in the roots of these genotypes (Vigh et al. 1985). However, a correlation between cold-hardiness and root lipid concentration was not shown in further studies with cucumber. Instead, results suggest that lipid composition plays a role in shoot tissue cold-tolerance (Bulder et al. 1991). Under heat stress, tomatoes grafted with heat-tolerant rootstocks showed increased vegetative growth and reduced chlorophyll fluorescence, indicating an increased tolerance to environmental stress. Reproductive development such as pollen production and fruit set was unaffected (Abdelmageed, Gruda, and Geyer 2004). Similarly, the accumulation of phenolics was reduced when tomatoes were grafted onto heat-tolerant rootstock, indicating the importance of this technique for managing heat stress (Rivero, Ruiz, and Romero 2003). In either case, the use of heat or cold-tolerant resistant rootstock can lead to the extension of the growing season in either direction, resulting in better yield and economic stability through the year.
Crop Productivity Enhancement

Although the vegetable grafting is typically associated with reduction of disease and/or abiotic injuries, yield is often increased without the presence of these identified stressors. Yields were increased by grafting in watermelon (Ruiz and Romero 1999; Yetisir and Sari 2003), and similar results have been found in cucumber (Pavlou, Vakalounakis, and Ligoxigakis 2002). In tomato, yield increases have shown how a vigorous root system in non-infested soils can lead to increased crop productivity (Upstone 1968). Yield increases were seen in eggplant as well, even without the presence of soilborne pathogens. Grafting eggplant onto wild Solanum rootstock showed significant yield increases as compared to self-grafted controls (Ibrahim et al. 2001; Rahman et al. 2002). In greenhouse production, eggplant grafted onto tomato rootstock showed improved yields as a result of increased fruit size and number compared to non-grafted controls and those with eggplant rootstock (Passam, Stylianou, and Kotsiras 2005). In tomato, increases in fruit yield are typically a result of increased fruit size (Augustin, Graf, and Laun 2002; Pogonyi et al. 2005). Fruit quality, as indicated by soluble solids, is reduced among grafted treatments in tomato. Possible explanations for this are that the improved productivity and subsequent yield quantity caused a decreased concentration of the main fruit components (Augustin, Graf, and Laun 2002; Pogonyi et al. 2005). In a similar way, osmotic stress caused by saline soils can increase fruit quality by increasing soluble solids (Colla et al. 2006; Fernandez-Garcia et al. 2004).

Conversely in melon, fruit quality, as indicated by fruit firmness, can be significantly increased in by grafting onto specific rootstock (Roberts et al. 2005).
Research has shown that possible mechanisms for increased yield are likely due to increased water and nutrient uptake among vigorous rootstock genotypes. Stomatal conductance was improved in tomato when grafted onto vigorous rootstock (Fernandez-Garcia et al. 2002). Nutrient uptake for macronutrients such as phosphorus and calcium were enhanced by grafting (Leonardi and Giuffrida 2006; Ruiz, Belakbir, and Romero 1996). Similarly, total N assimilation was increased as indicated by organic N and nitrate reductase activity within the leaf tissue of melon (Ruiz and Romero 1999). Among micronutrients, grafting melon with specific rootstock augmented Fe uptake as well as the subsequent translocation of this nutrient towards the shoot (Rivero, Ruiz, and Romero 2004). In tomato, although the rootstock showed greater capacity for Fe uptake and accumulation, this trend was not seen in the shoot, clearly indicating the importance of scion vigor in the uptake and metabolism of this micronutrient (Rivero, Ruiz, and Romero 2004). The overall importance of rootstock genotype was seen as photosynthesis rates were higher in tomato (Matsuzoe et al. 1993).

As a result of the increased vigor that commercial growers have seen from grafting with interspecific rootstocks in tomato, many growers in the Mediterranean have shifted to decreased planting densities (Besri 2003). These growers utilize production systems that can take advantage of enhanced vigor by training tomato vines into two heads or leaders. A crop productivity analysis of this method showed that although half as many plants were used per hectare, yields were increased by as much as 15% on a per hectare basis (Besri 2003). Furthermore, the extra production of these plants compensated for the added cost of transplants, and ultimately led to an increase of $5,610 per hectare (Besri 2003).
SECTION FOUR - RESEARCH OBJECTIVES

The objectives for this research program consist of the following:

1) To develop a protocol for grafting of tomato that is successful and adaptable for local growers.

2) To evaluate the use of grafting with bacterial wilt resistant rootstock for organic heirloom tomato production in naturally-infested fields. Furthermore, rootstock genotypes will be identified that may be used by commercial growers who wish to carryout grafting on the farm.

3) To understand how the use of disease resistant and highly vigorous rootstock affects crop productivity even under growing conditions that are not conducive to disease epidemics in the field.

4) To explore growing methods that can increase crop productivity on a per plant basis in order to reduce the additional costs associated with grafting and ultimately increase on-farm economic stability.

5) To investigate grafting as a major component of an integrated pest management program for organic or conventional tomato production.
LITERATURE CITED

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CHAPTER TWO – GRAFTING TO MANAGE SOILBORNE DISEASES IN HEIRLOOM TOMATO PRODUCTION

ABSTRACT

Organic heirloom tomato production in the southeastern United States is limited by foliar and soilborne disease, thermal stress, and weathered soil structure. Heirloom cultivars are highly marketable, but resistance to most diseases and tolerance of abiotic stressors is often poor. Diseases caused by soilborne pathogens such as *Fusarium oxysporum* f.sp. *lycopersici*, *Meloidogyne* spp., *Ralstonia solanacearum*, and *Verticillium dahliae* can lead to dramatic decreases in yield, and are difficult to manage especially under intensive organic production. Bacterial wilt (BW), caused by *Ralstonia solanacearum*, is particularly problematic due to the perseverance of the bacterium, and poor genetic resistance within horticulturally-acceptable cultivars. Fusarium wilt, caused by *F. oxysporum* f.sp. *lycopersici* is difficult to manage for heirloom growers as well, due to lack of genetic resistance. The purpose of this study was to investigate the use of resistant tomato rootstock for grafting to heirloom tomatoes for soilborne disease control. Field trials were implemented in 2005 and 2006 to observe disease incidence and crop productivity under conditions with and without pressure from soilborne plant pathogens. In naturally-infested soil, BW incidence for the heirloom cultivar ‘German Johnson’ in the non-grafted treatments was 79% and 75% in 2005 and 2006, respectively. However, German Johnson showed no symptoms of wilt in either year when grafted onto the resistant genotypes CRA 66 or Hawaii 7996. On-farm research trials with two additional rootstocks, Maxifort and Robusta, indicated that grafting treatment did not affect total yield under a typical organic management system with moderate to low disease pressure from soilborne pathogens. Fusarium wilt incidence was significantly
reduced as compared to non-grafted and self-grafted controls with the use of ‘Maxifort’ rootstock. In 2005 Maxifort’ rootstock increased yield of three heirloom cultivars when a twin row training system was implemented. In 2006, ‘Maxifort’ significantly increased yield in both standard and twin row training systems (P=0.005), but no difference was found across the two training systems. Grafting technology is a valuable management tool for reducing risk of crop loss due to soilborne diseases for organic heirloom producers as a major component in an integrated pest management program.
INTRODUCTION

Profitable organic tomato production is extremely challenging in the southeast as weathered soil structure, foliar and soilborne pathogen pressure, and mild winter temperatures can lead to a number of problems in the field. Furthermore, the development and importance of season extension combined with recurrent epidemics of foliar disease has forced many organic growers into high tunnels. These unheated greenhouses, or hoop houses, can provide season extension, enhanced fruit quality, and reduced foliar disease (Lamont Jr et al. 2003; Wittwer and Castilla 1995), but make long-term rotation difficult and inoculum from soilborne pathogens (*Fusarium oxysporum*, *Verticillium dahliae*, *Pyrenochaeta lycopersici*, *Phytophthora* spp., *Ralstonia solanacearum*, *Meloidogyne* spp.) can quickly build up to catastrophic levels.

Heirloom cultivars are regarded for their prolific coloration and exceptional flavor. These cultivars, however, are susceptible to a number of soilborne diseases, including bacterial wilt, and many heirloom growers need alternative pest management strategies that can be integrated into an organic production setting. US markets for these varieties are consumer-driven, and revenue generated from heirloom production is typically higher than that of standard field-grown fresh market fruit (Grassbaugh et al. 1999).

Bacterial wilt (*BW*), caused by *Ralstonia solanacearum*, is a problematic soilborne disease in many southeastern US crops including: tomato, potato, tobacco, and eggplant. This bacterium causes severe losses worldwide due to its distribution and unusually broad host range, including more than 50 plant families (Kelman 1998). The pathogen is a soil inhabitant and can easily move into non-infested areas through infested water and soil, and through infected plant material (Kelman 1998; Momol et al. 2005). The bacterium invades
plant roots through wounds or natural openings, colonizes the xylem tissue, and can cause total collapse of the plant within 2-3 days (McCarter 1991). The world population of \textit{R. solancearum} maintains considerable diversity, and has been separated into four biovars (Hayward 1964) and three races (Buddenhagen and Kelman 1964). In the US, biovar 1, race 1, is of greatest importance, as this race occurs endemically in the southeastern regions, causing southern bacterial wilt (McCarter 1991). Control of bacterial wilt in tomato is difficult. Crop rotation with a non-host crop may provide some control, but this measure is difficult due to the wide host range of the pathogen (Adhikari and Basnyat 1998; Lemaga et al. 2001; Melton and Powell 1991). \textit{R. solancearum} survives in the rhizosphere and the roots of symptomless weed species (Granada and Sequeira 1981; Umaerus 1992). Chemical fumigants for control of bacterial wilt in field production have had little success as methyl bromide, Telone-C35 and chloropicrin showed no efficacy in controlling this disease in several North Carolina field trials (Driver and Louws 2002).

Difficulty in breeding large-fruited, horticulturally-acceptable varieties with BW resistance has been well documented (Opena et al. 1990; Walter 1967; Wang et al. 1998), and small fruit size appears to be linked with resistance to the pathogen. A second challenge is that the complex diversity of pathogenic \textit{Ralstonia} strains has led to the development of resistant lines that are not durable over diverse geographic regions (Scott 1996). Tomato genotypes with bacterial wilt resistance originated from wild tomato, particularly \textit{Lycopersion esculentum} var. \textit{cerasiforme} and \textit{L. pimpinellifolium}. The genetic inheritance of bacterial wilt resistance is probably controlled multigenically with several modifier genes (Scott et al. 2005). In most cases, the resistance is not complete and breaks down to some extent when environmental conditions favor the pathogen (Danesh et al. 1994; Hartman and
Recently, a worldwide study evaluated 31 tomato genotypes from at least 14 resistance sources in 11 countries (Wang et al. 1998). Of all the evaluated genotype, Hawaii 7996 had the highest and most consistent survival percentages with a mean of 97% and a range of 85-100%.

Grafting has been essential in Asian horticultural production for eliminating bacterial wilt incidence in solanaceous crops (Oda 1999). In Brunei, bacterial wilt incidence is so high that tomatoes cannot be planted unless the soil is sterilized or resistant rootstocks are utilized (Peregrine and Binahmad 1982). The cultivation of modern grafted vegetable plants began in Korea and Japan at the end of the 1920’s when watermelon plants were grafted onto squash rootstock (Rivero et al. 2003). Since that time, vegetable grafting has been adopted throughout Asia and Europe. Currently, 81% and 54% of the vegetable acreage in Korea and Japan, respectively, uses grafting (Lee 2003). This cultural technique is mainly carried out for intensively managed crops grown in greenhouses or in tunnel production (Lee et al. 1998), but has become extremely popular recently in the Mediterranean region, where the use of grafting has been adopted as a major component of an integrated management strategy for soilborne disease and for increasing crop productivity (Besri 2001; Bletsos 2005; Giannakou and Karpouzas 2003; Pavlou et al. 2002).

There are a variety of techniques for grafting tomato. The most common method for commercial production is tube grafting or “Japanese top grafting.” Tube grafting takes place when the scion and rootstock are severed as seedlings and reattached with a small silicon tube or clip. Tube grafting has been adopted as the primary method for tomato grafting worldwide due to its high throughput production capability, and typical success rates of 85 to 90 percent (Oda 1995).
Many studies have shown that grafting can effectively complement other IPM strategies and decrease reliance on fumigation (Besri 2001; Bletsos 2005; Giannakou and Karpouzas 2003; Pavlou et al. 2002). This technology has been used to manage Verticillium and Fusarium wilt in melon and cucumber production systems in Japan, Korea, and Greece (Bletsos 2005; Ioannou 2001; Oda 1999). In tomato, grafting with resistant rootstock has been shown to be effective against *V. dahliae* (race 1), as well as corky root rot (caused by *Pyrenochaeta lycosersici*) (Bletsos 2005; Bradley 1968; Ioannou 2001). Grafting onto resistant rootstocks improved cucumber yield in the presence of *F. oxysporum f. sp. radicis-cucumerinum*, an emerging catastrophic disease in greenhouse production (Pavlou et al. 2002). In Morocco, grafting is used to control root-knot nematodes (*Meloidogyne* spp.) and other soilborne diseases in over 2000 ha of greenhouse tomato, melon and watermelon (Abdelhaq 2004; Besri 2001). Grafting with resistant rootstock has also been successful for cucumbers against root-knot nematodes in Greece (Giannakou and Karpouzas 2003). Eggplant production in the Mediterranean has been augmented by grafting onto resistant tomato rootstock for greenhouse production (Ioannou 2001), and eggplant rootstocks may provide resistance to root-knot nematodes for tomato production (Rahman et al. 2002). Tomato yellow leaf curl virus (ToYLCV), transmitted by *Bemisia tabaci*, may be effectively managed with the use of highly vigorous rootstock (Rivero et al. 2003).

Research has shown that grafting is also effective at overcoming abiotic stressors such as salinity, temperature extremes and excessive soil moisture. Rootstocks that are tolerant of high-saline soils could be instrumental in decreasing yield losses caused by salinity (Cuartero et al. 2006; Estan et al. 2005; Rivero et al. 2003). Grafting has been utilized in order to reduce the effects of flooding in areas where a wet season may occur
(Black et al. 2003). Rootstocks tolerant to thermal stress can affect crop productivity more than aboveground structures, and may be important for extending crop production cycles in vegetable production (Rivero et al. 2003). Many of the most economically important vegetable crops, such as tomato, squash, cucumber, and watermelon are highly sensitive to cold temperatures during their vegetative development and reproduction (Jackman et al. 1988). During winter cultivation, the rootstocks ‘Renova’ and ‘Esvier’ significantly promoted vegetative growth of the scion in cucumber production, but these effects were not seen during summer production (Zijlstra et al. 1994). Under heat stress, tomatoes grafted with heat-tolerant rootstocks showed increased vegetative growth and reduced chlorophyll fluorescence (Abdelmageed et al. 2004).

Although the use of grafted vegetables is associated with disease reduction and/or abiotic stressors, yield is often increased without the presence of these identified stressors. Yields can be increased in melon production even under optimum growing conditions (Ruiz and Romero 1999; Yetisir and Sari 2003). Furthermore, fruit quality, as indicated by fruit firmness, can be increased in watermelon by grafting onto certain rootstock (Roberts et al. 2005). In tomato, yield increases in fumigated soil have been seen (Upstone 1968). In most cases, increased fruit yield is a result of increased fruit size (Augustin, Graf, and Laun 2002; Pogonyi et al. 2005). Grafting eggplant onto wild Solanum rootstock also showed that yield was increased as compared to self-grafted controls (Ibrahim et al. 2001; Rahman et al. 2002). In greenhouse production, eggplant grafted onto tomato rootstock enhanced yields with increased fruit size and number as compared to non-grafted controls and those with eggplant rootstock (Passam et al. 2005). Similarly, yields were improved in cucumber as a result of grafting without the presence of known soilborne pathogens (Pavlou et al. 2002).
Possible mechanisms for increased yield are probably due to increased water and nutrient uptake by vigorous rootstock genotypes. Stomatal conductance was improved in tomato when grafted onto vigorous rootstock (Fernandez-Garcia et al. 2002). Nutrient uptake for macronutrients such as phosphorus and calcium was enhanced by grafting (Leonardi and Giuffrida 2006; Ruiz et al. 1996). Similarly total N assimilation was increased within the leaf tissue of melon (Ruiz and Romero 1999). Among micronutrients, grafting with certain rootstock for melon production augmented Fe uptake and subsequent translocation into the shoot. In tomato, certain rootstocks showed greater capacity for Fe uptake and accumulation, but this trend was not seen in the shoot, leading the authors to conclude scion vigor is also important in the uptake and metabolism of this micronutrient (Rivero et al. 2004). Photosynthetic rates were higher in tomato grafted onto wild eggplant genotypes (Matsuzoe et al. 1993).

In tomato, the increased vigor due to inter-specific rootstocks, has led to production systems with decreased planting densities (Besri 2003). These systems take advantage of enhanced vigor by utilizing plants with two heads or leaders. A crop productivity analysis of this alternative cultural method showed that although half as many plants were used, yields were increased by as much as 15% on a per hectare basis when a two-headed scion was grafted with ‘Maxifort’ rootstock (De ‘Ruiter Seed Co, the Netherlands). The extra production of these plants led to an increase of $5,610 US per hectare (Besri 2003).

In the southeastern US, many heirloom tomato growers encounter soilborne diseases. Growers are also interested to learn about the overall productivity of rootstock and alternative management practices that may improve the economic feasibility of this technology even under low disease pressure. There is a need to determine the efficacy of
resistant rootstock to manage BW in naturally-infested soils in the SE-USA. We hypothesize that grafting with resistant genotypes used as rootstock could be an effective management tool for growers of heirloom tomatoes. Therefore, the purpose of this study was to evaluate resistant rootstock genotypes in heirloom tomato production in field soils naturally infested with *R. solanacearum*. Furthermore, commercially-available rootstocks were tested in an organic production setting, with low disease pressure, under standard and alternative training systems to determine the utility of grafting for organic heirloom tomato for production in the southeastern US.
MATERIALS AND METHODS

Bacterial Wilt Trials:

Grafted transplants were produced in greenhouse facilities on the NCSU campus using the tube grafting technique (Rivard and Louws 2006). In all experiments, ‘German Johnson’ was used as the susceptible heirloom cultivar, and CRA 66 and Hawaii 7996 were utilized as resistant rootstocks. The four grafting treatments included non-grafted, self-grafted, CRA 66 rootstock, and Hawaii 7996 rootstock. Plants were grafted at 3 weeks, and subsequent field planting occurred two weeks after grafting had occurred. All field trials were planted in a randomized complete block design and included four replications. Seven plants per plot were transplanted in 2005, and six plants per plot were in 2006. Sites with bacterial wilt pressure were on non-organic farms. Thus, cultural management was consistent with typical commercial production in NC including the use of fungicides and insecticides for disease and insect control. Transplants were set into a 15 cm high, 75 cm wide raised bed plasticulture system with 1.5 m row spacing. Black plastic mulch and drip irrigation was employed and a stake-and-weave cultural management was used.

Pender County Trials: Field trials were conducted in Pender County, NC (37°25’8.18” N, 72°26’5.36” W) on a farm with a history of endemic populations of R. solanacearum. Soil type consisted of a Norfolk sandy loam (pH=6.0). Blended preplant fertilizer (8-3-20) was applied at 672 kg/ha, and two CaNO₃ fertigation supplements were supplied at 67 kg/ha on 20 May and 15 June. The trial was established and planted on 10 May 2005, and bacterial wilt incidence was monitored on 8 June, 6 July, 13 July, and 20 July. For each plot, disease incidence was scored based upon the number of plants per plot that displayed onset of total wilt. Diagnostic symptoms of bacterial wilt included a total loss in turgor pressure and no
signs of yellowing or necrosis. Stem segments of wilted plants (2.5 cm) were excised, surface sterilized, and examined for bacterial streaming from the xylem tissue. Bacteria were collected from the streaming assay, and streaked for single colony forming units on Kelman’s TZC semi-selective medium (Kelman 1954). Change in pigmentation within bacterial colonies grown on the medium indicated the presence of virulent strains of *R. solanacearum*. Tomato spotted wilt virus (TSWV) was also observed based upon the appearance of diagnostic symptoms within the field. Typically, TSWV symptoms appear as bronzing of the leaves and stunting at the apical meristem, as well as spots on the fruit (Zitter 1991). Verification of TWWV particles was assessed with Agdia TSWV ImmunoStrips® (Agdia, Elkhart, IN). Tomato fruit harvesting was carried out on July 13, 20, 27, and August 2. Fruit were graded as marketable or non-marketable, and fruit number and weight were recorded. All results were analyzed using ANOVA (PlotIt, Scientific Programming Enterprises, Haslett, MI) with mean separation by using a F-protected least significant difference (LSD).

In 2006, the trial in Pender County was planted on 6 June 2006. Bacterial wilt incidence was monitored on 20 June, 11 July, 18 July, 25 July, and 7 August. The late planting time and excessive moisture from two hurricane events led to poor fruit set. Yields were not recorded for this trial and TSWV did not occur.

**Rowan County Trial:** The Rowan County trial (35°37’2.20” N, 80°39’5.92” W) was planted on 10 August, 2005, typical of a fall planting time in the piedmont growing region of NC. The trial was established on land with a history of bacterial wilt but wilt due to *R. solanacearum* did not occur. Soil type consisted of Cecil sandy clay loam (pH =6.4). Blended preplant fertilizer (8-3-20) was applied at 840 kg/ha, and CaNO₃ fertigation was
supplied at 84 kg N/ha on 25 August. Two harvests were made on 10 and 19 October, and plant growth data was observed to determine the effect of grafting with CRA 66 and Hawaii 7996 rootstock in the absence of disease pressure. One plant was destructively sampled from each plot across the four replications (n=4) on 19 October. The root system of each plant was visually scored for root vigor on a scale from 1-5 (1 = highly vigorous with many thick and fine roots, 2 = vigorous with thick and fine roots, 3 = less vigorous with few thick and many fine roots, 4 = few thick and fine roots, 5 = few fine roots). Plant height was measured and shoot biomass was dried for 72 hours at 70 C and dry weight was determined. All data were analyzed as described previously.

**Organic Crop Productivity Trials:**

**On-farm Trials:** In 2006, duplicate experiments were implemented to determine the efficacy of using highly vigorous rootstock genotypes ‘Maxifort’ and ‘Robusta’ to increase crop productivity for organic heirloom tomato production. The two field sites were chosen due to the long history (20+ years) and vast expertise of organic culture by the growers. Field sites were located at Peregrine Farms in Alamance County, NC (35°52’23.62” N, 79°15’ 29.31” W) and Maple Spring Gardens, in Orange County, NC (36°13’58.06” N, 79°11’3.36” W). Crop rotation with non-solanaceous vegetables and cut flowers was practiced at each site. Diseases caused by soilborne pathogens had not been observed as a major concern in tomato at either site.

The heirloom cultivar, ‘German Johnson’, was used for non-grafted and self-grafted controls and as scion for grafted treatments at both sites. The four grafting treatments were non-grafted, self-grafted, ‘Maxifort’ rootstock, and ‘Robusta’ rootstock. Each treatment consisted of seven plants per plot and four replications in a randomized complete block
design. Tube-grafted and non-grafted transplants were produced on the NCSU campus using the tube-grafting technique (Rivard and Louws 2006). Transplants were set 2 weeks after grafting had occurred. Plant age was approximately 5 weeks, and plants were trained to a vertical trellising system.

The Alamance County trial was planted on 22 May 2006. Soil type consisted of Efland silt loam (pH = 6.0). Prior to planting, a rye/hairy vetch cover crop was incorporated into the soil. Preplant nitrogen was supplied through a feathermeal application at 111 Kg N/ha. Plant spacing was set at 46 cm as per grower requirements, and transplants were planted into a cultural system typical for the grower. Straw mulch was used along with drip irrigation. A vertical trellis system was built using steel posts and 1.3 m wide wire mesh with 10 cm x 10 cm spacing. Suckering was carried out up to the first fruit hand, and vines were attached to the trellis system with a “fast tapener” which bound the vines onto the metal wire mesh with vinyl tape. Harvesting was carried out on 25 July, 1, 9, 14, 21, 24, 30, August, and 4, 15 September. Fruit were graded as marketable and non-marketable, and fruit number and weight were recorded. Fusarium wilt, caused by *F. oxysporum* f.sp. *lycopersici*, occurred unexpectedly throughout the field. Disease incidence was monitored as number of symptomatic plants per plot on 29 June, 12, 21 July, 9, 21, and 5 September. Symptomatic plants were identified by yellowing of leaf tissue throughout the aboveground biomass, and discoloration of the xylem tissue. Stem segments from symptomatic plants were excised (1 cm), surface sterilized in 10% bleach solution, rinsed with distilled water, and placed on acidified potato dextrose agar. A disease progress curve was plotted, and an AUDPC value was calculated for each treatment. Harvest yields and disease incidence were analyzed using ANOVA (PlotIt, Scientific Programming Enterprises, Haslett, MI)
The Orange County trial was planted on May 23rd, 2006. Soil type consisted of an Appling sandy loam (pH = 6.8). A winter rye/hairy vetch cover crop was incorporated, and preplant fertilizer applications consisted of feather meal at 111 kg N/ha and sulfate of potash at 140 kg K/ha. Plant spacing was set at 56 cm as per grower requirements. Transplants were set into a 15 cm high, 75 cm wide raised bed plasticulture system with 1.5 m row spacing and drip irrigation. Suckering was carried out up to the first fruit hand, and plants were managed using a vertical trellis system. Vines were trained to a single 2 m stake throughout the season with nylon twine. Harvesting was carried out on 21, 28, 31, July, 4, 7, 10, 13, 17, 22, 25, 28, 30, August and 15 September.

**Plant Training Systems:**

**CEFS Trial 2005:** A non-replicated plot was implemented within the organic small farm unit at the Center for Environmental Farming Systems (Goldsboro, NC; 35°23′30.97″ N, 78°1′42.28″ W). The small farm unit educates local sustainable and organic practices through internship and apprentice programs, farm tours, and extension workshops, and has been managed organically since 1996. All tomato transplants were produced and tube-grafted at the NCSU Phytotron. Scion varieties included ‘German Johnson’, ‘Cherokee Purple’, and ‘Jubilee’. Each scion variety received grafting treatments including: non-grafted, self-grafted, and ‘Maxifort’ rootstock. Soil type consisted of a sandy loam (pH = 6.5). Preplant nitrogen was supplied through the application of soybean meal at 111 Kg N/ha. Ten cm high, 120 cm wide raised bed plasticulture with 1.8 m row spacing was utilized. Reflective plastic mulch was used, and plant training methods included standard field culture, commonly referred to as the California/Florida stake-and-weave method as well as a two-headed culture, whereby a twin row of stakes ran down the length of the bed. This
system utilized similar management to that of the standard culture, but instead of a single row of stakes running down the length of the bed, a twin row of stakes was used, spaced 46 cm apart. For transplants that were to be placed in the twin row culture system, the meristematic tissue at the top of the plant was pinched out, inducing the apical formation of two “heads” that could be trained accordingly. At 5 weeks, two weeks after post-graft healing and acclimation had occurred, pinching was carried out among transplants that would go into the twin row system, and all plants were transferred into 10 cm pots, where they grew for 10 days until field planting. Transplants were planted in the field on 19 May 2005, and soil moisture was maintained through drip irrigation.

Treatments were placed into a 1.5 by 88 m row similar to a split-split plot design, whereby the first main plot consisted of the two training systems. The sub-plots consisted of the three scion treatments. Within the scion treatments sub-sub-plots were designated by the three grafting treatments (non-grafted, self-grafted, ‘Maxifort’ rootstock) and randomized within each scion mainplot. A schematic representation of the 18 treatments within the experimental layout can be seen in figure 1.

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**Figure 1** – Schematic representation of experimental design at 2005 CEFS observational trial.
Each plot consisted of five plants and spacing within the row was 61 cm. Harvesting was carried out on 21, 26, 30, July and 4, and 11 August. The number and weight of fruit were recorded.

The yield impact of grafting upon each scion cultivar \((Y_{sx})\) was calculated by averaging the normalized yield effect of the two training systems (Std and TR) for a given scion. Within each training system, yield was normalized by dividing the fruit weight for the grafting treatment \((x)\) of interest, by the fruit weight of the non-grafted treatment (non) with the same scion cultivar. These values for the two training methods (Std and TR) were averaged for each scion \((s)\) and grafting treatment \((x)\) of interest. For training systems (Std and TR), scion of interest \((s)\), and grafting treatment of interest \((x)\),

\[
Y_{sx} = \frac{\left(\frac{\text{Std Yield}_x}{\text{Std Yield}_{\text{non}}}\right) + \left(\frac{\text{TR Yield}_x}{\text{TR Yield}_{\text{non}}}\right)}{2}
\]

To determine the yield impact of grafting upon each training method \((Y_{tx})\), a normalized average across the three scion cultivars (GJ, CP, J) was calculated for each training system. In this case, the grafting treatment of interest \((x)\) was normalized as compared to the non-grafted (non), standard (Std) culture control with the same scion. These values for the three scion cultivars (GJ, CP, J) were averaged for each training system \((t)\) and grafting treatment \((x)\) of interest, indicating the average yield impact that grafting has upon training system. For a grafting treatment of interest \((x)\) and training method of interest \((t)\):

\[
Y_{tx} = \frac{\left(\frac{\text{GJ Yield}_{X, t}}{\text{GJ Yield}_{\text{Non, Std}}}\right) + \left(\frac{\text{CP Yield}_{X, t}}{\text{CP Yield}_{\text{Non, Std}}}\right) + \left(\frac{\text{J Yield}_{X, t}}{\text{J Yield}_{\text{Non, Std}}}\right)}{3}
\]
CEFS 2006: In 2006, a replicated alternative training systems trial was planted within the organic small farm unit at the Center for Environmental Farming Systems as above. All tube-grafted tomato transplants were produced at the NCSU Phytotron (Rivard and Louws 2006). Preplant fertilization was supplied through an application of soybean meal at 111 Kg/ha. A raised bed plasticulture system was used as before with reflective mulch, and plant training systems included California/Florida stake-and-weave system as the standard culture method, and the twin row method, as previously described. German Johnson was the sole scion type and grafting treatments consisted of: non-grafted, self-grafted and ‘Maxifort’ rootstock.

The experimental layout was a replicated split plot design. Main plots consisted of plant training systems and the subplots comprised of the three grafting treatments. Four replications were planted into four 60 m rows, and main plots were randomized within each replication and subplots randomized within the main plots. Each plot consisted of six plants with 46 cm spacing between plants. The trial was established and planted on 5 July 2006, and fruit was harvested 12, 21, 29, September and 9, 13, 20, and 25 October. Fruit number and harvest yields were analyzed as previously described.
RESULTS

On-Farm Trials – Bacterial Wilt: In the Pender County trial, high levels of bacterial wilt incidence occurred in ‘German Johnson’ for the non-grafted and self-grafted treatments in 2005 and 2006 (Figure 2). Disease incidence at the end of the season had reached 75% in the non-grafted plots in 2005 and 79% in 2006. The self-grafted plants had an intermediate level of wilt incidence in 2005 of 49% and a high incidence of 83% in 2006. No symptomatic plants were identified when German Johnson was grafted onto the CRA 66 or Hawaii 7996 rootstock in 2005 or 2006. In 2005, cumulative TSWV incidence ranged between 20% and 75% across all treatments (Figure 3). TSWV incidence was higher in non-grafted treatments as compared to CRA 66 (P=0.05), but no other treatment effects were found. Marketable yields were not affected by grafting treatment, but cumulative total yields in 2005 were 104% higher in the Hawaii 7996 rootstock treatments as compared to the non-grafted controls (P=0.04; Figure 4). At first harvest, total yield of CRA 66 and Hawaii 7996 were 300% and 243% higher than non-grafted treatments, respectively (P=0.05), and were significantly higher than self-grafted treatments.

Since bacterial wilt did not occur in the Rowan County trial, an assessment of yield and plant growth in the absence of disease pressure was carried out. Rootstock did not significantly impact total yield or number of fruit harvested (Figure 5). CRA 66 rootstock had superior root vigor as compared to all other treatments (P=0.01, Figure 6A) and resulted in enhanced plant height (P=0.09) and shoot biomass (P= 0.05) as compared to the non-grafted treatment (Figure 6B).

On-Farm Trials – Organic Crop Productivity: In the Alamance County trial, symptoms of Fusarium wilt (caused by *F. oxysporum* f.sp. *lycopersici*) were observed 50 days after
transplanting for non-grafted and the self-grafted controls (Fig 7A). Disease incidence in the non-grafted and self-grafted controls had final incidences of 46% and 50%, respectively (Figure 7A). No symptoms of Fusarium wilt developed when German Johnson was grafted onto ‘Maxifort’ rootstock while plants on Robusta rootstock had a terminal incidence of 29% (Figure 7A). For plants on Robusta rootstock, Fusarium wilt increased from less than 10% at 80 days after transplanting, to almost 30% 10 days later when most of the crop had been harvested. Likewise, Fusarium wilt incidence increased about 20% in the same 10-day period for German Johnson and the self-grafted controls. Over the course of the season, the area under disease progress curve for Fusarium wilt showed a significant benefit of grafting with ‘Maxifort’ and ‘Robusta’ rootstock compared to non-grafted and self-grafted controls (P=0.002, Figure 7B). ‘Maxifort’ treatments showed the lowest AUDPC values, and ‘Robusta’ showed and intermediate value, but there was no significant effect between the two (Figure 7B). Cumulative marketable yield and total yield showed no significant treatment effects (Figure 8). In the Alamance County trial, crop productivity on ‘Robusta’ rootstock was greater than all other treatments early during the season (P=0.05). Plants on ‘Maxifort’ rootstock showed reduced yield as compared to ‘Robusta’ during this time, but cumulative marketable and total yields were equivalent 91 and 84 days after transplanting, respectively (P=0.05, Figure 8). In the Orange County trial, yields regardless of treatment were nearly identical for harvests up to 85 days after transplanting but by 90 days after transplanting the ‘Robusta’ rootstock treatment had reduced total fruit weight as compared to the self-grafted and ‘Maxifort’ treatments (P=0.05, Figure 9).

**CEFS Trial 2005** – The heirloom scion cultivar affected the efficacy in grafting to ‘Maxifort’ rootstock to improve total yield (Figure 10A). Total yield was increased for the
‘Maxifort’ treatments with ‘Cherokee Purple’ scion. For the other two scions tested, ‘German Johnson’ and ‘Jubilee’, increases could be seen in the ‘Maxifort’ treatments as compared to the non-grafted controls, but not when compared to the self-grafted treatments. Training system had a dramatic effect on yield in 2005 (Figure 10B). Grafting had little effect within the standard training system. However, large increases in yield were seen with the implementation of the twin-headed training method. Across the three heirloom cultivars, the twin-heading method increased yield in non-grafted treatments by 118% as compared to non-grafted controls in the standard training system. Compared to the non-grafted treatments under the standard training system, the use of ‘Maxifort’ rootstock, increased total yield by 287%.

**CEFS 2006:** In 2006, ‘Maxifort’ rootstock significantly increased tomato yield in both the standard and twin row training systems in comparison to non-grafted or self-grafted controls (P=0.005; Figure 11). No difference in yield was found between the two plant training systems and no interactions were observed between training system and grafting treatment.
DISCUSSION

Grafting has two distinct functions. First, resistant rootstock can be deployed to limit risk of crop losses from soilborne pathogens. Second, some rootstocks have been selected for potential to increase plant vigor and crop yield. In this study, we evaluated both functions. In fields with a history of bacterial wilt pressure, CRA 66 and Hawaii 7996 completely controlled bacterial wilt when used as rootstock for the susceptible heirloom cultivar ‘German Johnson’. To our knowledge this is the first report of grafting to manage bacterial wilt in the United States.

Tomato growers in the SE USA do not have effective tools to manage this serious soilborne pathogen. Fumigation is not effective (Driver and Louws 2002) and lines with bacterial wilt resistance tend toward small fruit size or incomplete control (Scott et al. 2005). These problems can be especially acute for organic growers who prefer heirloom tomato cultivars. Oftentimes, organic farmers abandon land that has a history of BW pressure. The resilient bacterial wilt resistance shown in this study by CRA 66 and Hawaii 7996 rootstocks indicates the importance of grafting to manage bacterial wilt in severely infested soils. CRA 66 has been identified as a resistant rootstock genotype for grafted tomato production in India and Germany (Grimault et al. 1994; Tikoo et al. 1979). A worldwide survey of resistant genotypes showed that Hawaii 7996 is most effective against the various strains of *R. solanacearum* worldwide (Wang et al. 1998). In this study, it was evident that both CRA 66 and Hawaii 7996 genotypes displayed extremely high resistance to endemic populations of *R. solanacearum* in eastern North Carolina. Increased root vigor and plant growth by the CRA 66 rootstock indicates that this genotype may display enhanced vigor, but, this may be a tendency towards more vegetative growth by CRA 66 rootstock. The Pender County trials
demonstrate the efficacy of the resistance, but crop yield data are not reliable. In 2005, high
levels of TSWV occurred, creating concerns about the relevance of crop yields. In 2006,
yield data collection was limited by hurricane events. However, in a third repetition of this
experiment in Rowan County, no soilborne or foliar diseases were evident, and yields were
unaffected by the use of resistant rootstocks. The Pender County and Rowan County
experiments taken together suggest these rootstocks completely mitigate the risk of BW
incidence and do not threaten crop productivity.

A surprising discovery in the 2005 Pender County trial that could be productive
research in the future was the dramatic impact CRA 66 had on TSWV incidence. Tolerance
to viruses, moderated by rootstock with increased vigor has been observed for ToYLCV
(Rivero et al. 2003), but never suggested for TSWV to date. TSWV incidence did not occur
in 2006, so the robustness of this rootstock to limit TSWV is not clear, but is worth
evaluating in the future.

The unexpected incidence of fusarium wilt on the Alamance County organic farm
reiterates the value of resistant rootstock to reduce risk in heirloom production. ‘Maxifort’
carries F1 and F2 resistance, and ‘Robusta’ has F2 resistance, leading us to predict the
fusarium race present in the field was race 1. ‘Maxifort’ offered complete control and
‘Robusta’ delayed the onset of wilt and limited the final incidence. To our knowledge, this is
the first report of fusarium wilt management in the field through grafting in tomato. The
reduced risk of fusarium wilt can be of great value to organic and conventional growers of
heirloom cultivars. Major resistance genes have been deployed in modern hybrid cultivars to
manage this disease (Jones 1991). However, because heirloom varieties do not carry genetic
resistance to fusarium wilt, grafting could be a primary component to integrate these genetic control mechanisms into an heirloom production system.

In the case of our experiment, the onset of wilt occurred later during plant development, and disease incidence did not have a dramatic effect on yield. However, the negative impact of fusarium wilt upon tomato yield in severely-infested soils is well documented in the literature (Fuchs et al. 1999; Khan and Khan 2002).

The use of host resistance as a disease management tactic often leads to a trade-off, resulting in a reduction in crop productivity by resistant genotypes when there is an absence of disease (Bergelson and Purrington 1996; Brown 2002). Growers may be willing to accept this trend due to enhanced long-term economic stability with the use of resistant cultivars. Because grafting does not negatively impact yield, there is no trade-off in reduced yields. The trade-off associated with the implementation of grafting is imposed by an increase in transplant production cost.

The main goal of organic and other heirloom tomato growers is to reduce risk of crop failure without compromising yield and preferably increasing yield. In the two organic on-farm trials, the ‘Maxifort’ and ‘Robusta’ did not consistently impact total yield. In one trial, ‘Maxifort’ tended to delay early fruit set whereas ‘Robusta’ increased early fruit set as compared to all other treatments. In contrast, ‘Robusta’ was not as effective in sustaining late season yield in the Orange County trial. The dynamics of rootstock on early vs. late season productivity is an important consideration for growers seeking to sell on an early or late market. Both ‘Robusta’ and ‘Maxifort’ also contain the Mi gene, conferring tolerance to root-knot nematode, and therefore should offer reduced risk of crop failure due to this important soilborne plant pathogen, as well as selected fusarium races. In contrast,
‘Maxifort’ generated the highest yields as compared to self- and non-grafted treatments in the CEFS experiments in 2005 and 2006. The reason for yield benefit in the organic system at CEFS compared to the on-farm experiment is not clear and is a productive question for the future research.

A key question in the mind of local growers would relate to the economics of grafting. Clearly, if major crop losses would recur due to soilborne disease, then grafting would be viable. Alternatively, growers could modify their plant training systems to capture the benefits of enhanced plant vigor afforded by the rootstock. This is done in commercial soil-based tunnel systems in the Mediterranean (Besri 2003). We evaluated the utility of a double row training system. This training system functionally doubles the plant surface area arising from a single rootstock. This training system increased total yield in 2005, but not in 2006. A productive component of future research is to explore plant training systems that maintain or enhance yield on a per plant basis leading to reduced rootstock density, and therefore reduced economic constraints for a grafted tomato production system.

Grafting may be an effective IPM tool for growers that face heavy disease pressure from soilborne pathogens and are constrained by market demands to supply high-value heirloom fruit. Growers who have eliminated or who are limited to short crop rotations, like those operating in tunnels, may benefit greatly from this emerging technology. Furthermore, growers that are transitioning production areas from conventional to organic management often face elevated pest pressure (Zinati 2002). In addition to bacterial wilt and fusarium wilt, root-knot nematodes can be particularly problematic in these situations (McSorley 2002), and resistant rootstock may be an important component for heirloom production.
Figure 2 - Bacterial wilt disease incidence of grafted and non-grafted heirloom tomato cv. German Johnson in naturally-infested fields during A, 2005 and B, 2006 (Pender County, NC). Incidence for both years was analyzed by mean separation with a protected LSD (P=0.05).
Figure 3 – Tomato Spotted Wilt Virus incidence of grafted and non-grafted heirloom cv. German Johnson tomato in 2005 (Pender County, NC). Incidence was analyzed by mean separation with a protected LSD (P=0.05).
Figure 4 - Cumulative marketable (A) and total (B) fruit yield of grafted and non-grafted heirloom tomato cv. German Johnson in bacterial wilt-infested fields (Pender County, NC). Results for marketable yield were not significant. First and last harvest cumulative harvest yields were analyzed by separation of the mean with a protected LSD value (P=0.05)
Figure 5 - Fruit number and weight harvested from grafted and non-grafted heirloom cv. German Johnson tomato in the absence of bacterial wilt symptoms (Rowan County, NC).
Figure 6 - Root vigor (A) and plant shoot DW and height (B) of grafted and non-grafted heirloom cv. German Johnson tomato in non-infested soils (Rowan County, NC). Results were analyzed by mean separation with a protected LSD value (P-value given).
**Figure 7** - Disease incidence (A) and AUDPC (B) for fusarium wilt of grafted and non-grafted heirloom tomato cv. German Johnson in naturally-infested soils ( Alamance County, NC). Results were analyzed by mean separation with a protected LSD (P=0.05)
Figure 8 - Cumulative marketable (A) and total (B) fruit yield of grafted and non-grafted heirloom tomato cv. German Johnson (Alamance county, NC). Results were analyzed by mean separation with a protected LSD (* denotes P=0.09, all others given).
Figure 9 - Cumulative marketable (A) and total (B) fruit yield of grafted and non-grafted heirloom tomato cv. German Johnson (Orange county, NC). Results analyzed by mean separation with a protected LSD (P-values given).
Figure 10 – Normalized scion effect (A) and training effect (B) for total fruit yield of grafted and non-grafted tomato heirloom tomato (CEFS, 2005). Error bars represent standard error ($[s^2/n]^{1/2}$) about the mean.
Figure 4.8 - Main Effects of Grafting and Twin Row Training System: CEFS, 2006

Figure 11 - Total fruit yield of grafted and non-grafted tomato cv. German Johnson for standard and twin-head training systems (CEFS, 2006)
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Agriculture in America is changing. Consumer demand for organically-raised fruit and vegetables have significantly increased organic production in the US. Furthermore, elevated energy prices and the reappearance of the farmer’s market, has made small-scale, local, and sustainable agriculture ever more popular. Among the many crops that these growers bring to market, heirloom tomatoes make up a substantial portion of on-farm revenue. An unfortunate dilemma exists for these growers as their crop production system relies heavily upon host resistance, but a major economic staple, heirloom tomato, has none. Grafting with disease resistant and highly vigorous rootstock may be an important component in an integrated approach to manage soilborne disease and improve yields for these growers.

Grafting with resistant rootstock eliminated bacterial wilt disease incidence, even in severely infested fields (Figure 1). ‘Maxifort’ rootstock completely controlled fusarium wilt, and ‘Robusta’ rootstock was able to provide intermediate control of this disease (Figure 7). Finally, ‘Maxifort’ rootstock was able to functionally compensate for lack of crop rotation under disease pressure from verticillium wilt (see Appendix B). Currently, many growers in the Mediterranean that utilize grafting carryout two-headed culture (Besri 2005). Because yields were not significantly affected under little disease pressure unless grafting was incorporated with alternative training systems, our results coincide with this trend. In order to optimize the economics of grafting, full utilization of additional vigor must be carried out through cultural management.

As seen in our studies, grafting is probably most beneficial under severe pressure from soilborne disease or abiotic stress (Rivero et al. 2003). The greatest beneficiaries of this
technology may be growers who carryout soil-based production in tunnels and greenhouses. Innovative production systems like high tunnels or haygrove tunnels have recently been promoted to manage foliar disease, increase fruit quality, and extend the growing season (Lamont Jr. et al. 2003; Wittwer and Castilla 1995). In North Carolina, this trend is evident as organic growers face recurrent management problems associated with early and late blight, caused by *Alternaria spp.* and *Phytophthora infestans*. In tunnel or greenhouse production, crop rotation is typically not available, and environmental conditions may be less than ideal. Grafting with disease resistant rootstock may be able to compensate for a lack of rotation in these systems as rootstock can withstand severe pressure from soilborne pathogens (Besri 2001; Lee et al. 1998). Furthermore, season extension can be augmented by rootstocks that are tolerant of excessive thermal stress (Rivero et al. 2003), leading to increased farm-gate income and greater economic stability throughout the year. A PhD research program has been initiated by the author including a systems evaluation of high tunnel production that incorporates grafting with disease resistant rootstock. This information will be essential to organic growers, as the combined use of these cultural practices could further enhance sustainable production in the southeast.

Interestingly, self-grafted treatments often resulted in intermediate yields or disease resistance (Figures: 3, 4, 6, 9B, 10). This indicates the importance of a physiological investigation of grafting, to determine how this process affects plant growth and development. Specifically, current research has shown an elevation of several defense genes as a result of wounding (Schilmiller and Howe 2005). Proteinase inhibitors were the first of these to be cited (Green and Ryan 1972). A real-time PCR protocol has been developed to
characterize the induction of this gene as a result of grafting, and this study will be elaborated upon in future PhD studies by the author.
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Besri, Mohammed. 2001. New developments of alternatives to methyl bromide for the control of tomato soilborne pathogens in covered cultivation in a developing country, Morocco. 2001 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions November 5-8, 2001, at San Diego, California, USA.


APPENDIX A – GRAFTING FOR DISEASE RESISTANCE IN HEIRLOOM TOMATOES

authored by:

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&
Frank Louws

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North Carolina Cooperative Extension Service

College of Agriculture and Life Sciences
Raleigh, NC 27695
Grafting for Disease Resistance in Heirloom Tomatoes

Grafting is a simple technique that growers can use to increase soilborne disease resistance in tomatoes without chemical fumigants or pesticides.

Heirloom tomato cultivars lack genetic disease resistance and are particularly susceptible to epidemics in the field. Growers interested in this niche market, however, are not willing to give up the high-quality fruit that they deliver. Grafting can be used to unite the soilborne disease resistance and enhanced vigor of hybrid tomato cultivars with the high fruit quality of heirloom varieties.

Grafting: An Old Technology with a New Technique

Grafting vegetables to manage soilborne disease is a simple process. The below-ground portion of a plant—the rootstock—is chosen for its genetic ability to resist or tolerate soilborne disease. The above-ground portion of the plant—the scion—is chosen based on fruit quality.

“Japanese top-grafting” or “tube grafting” is a new technique that is especially popular for tomato production in commercial greenhouses worldwide because the process is very fast and large numbers of grafted seedlings can be managed easily throughout the healing process (Figure 1). Each seedling is severed just above the cotyledon. The above-ground portion (scion) of the heirloom variety is secured to the root system (rootstock) of the disease-resistant seedling. Once the grafted transplants heal, they can be planted in the field and managed according to the grower’s production system.

Although vegetable grafting is relatively new, it relies on an old principle. Grafting has been used in the horticultural industry for woody species, such as apples and grapes, for centuries. The first use of vegetable grafting to reduce soilborne disease occurred in the early 1900s to diminish fusarium wilt on watermelons. More recently, this technique has been used in plant production systems when the genetic trait for disease resistance in a crop is closely linked to a negative quality, such as small fruit size. For example, grafting is often used to reduce bacterial wilt (caused by Ralstonia solanacearum) in tomatoes. This disease complex is particularly difficult to manage due to its wide range of hosts and its ability to persevere through long crop rotations. In addition, tomato cultivars that are resistant to bacterial wilt are typically not capable of producing large, marketable fruit. The worldwide use of grafting with resistant rootstock has significantly decreased bacterial wilt incidence while keeping fruit quality high, even in severely infested soils.

Figure 1. Silicon tube-shaped clips are used for “Japanese top grafting.”

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Benefits
Grafting can be used where growers need to increase soilborne disease resistance and keep fruit quality high enough to compete in fresh-produce markets. Grafted tomato transplants are able to combine the high-quality fruit of heirloom cultivars with the disease resistance, stress tolerance, and vigor of modern rootstock cultivars. (For further details on how grafting has been used to increase crop production worldwide, see “Recommended Reading” at the end of this publication.)

Disease resistance
Researchers around the world have demonstrated that grafting can be effective against a variety of soilborne fungal, bacterial, viral, and nematode diseases. It has been used to eliminate verticillium and fusarium wilt in tomato and cucurbit production systems in Japan, Korea, and Greece. In New Zealand, it has been used to reduce levels of corky root rot. In Morocco and Greece, grafting is used to control root-knot nematodes (Meloidogyne species) in both tomatoes and cucurbits. Researchers have proposed using grafted plants instead of methyl bromide to manage soilborne disease in these regions of the world.

Grafting has been essential in Asian horticultural production for eliminating bacterial wilt incidence in solanaceous crops, such as tomatoes and peppers. It has also been used in tropical environments, like the South East Asian kingdom of Brunei, where bacterial wilt incidence is so high that tomatoes cannot be planted unless the soil is sterilized or resistant rootstocks are used. In India, wilt-resistant rootstocks were used in one experiment to reduce bacterial wilt in tomatoes. By the end of the season, none of the control plants had survived, while 100% of the grafted plants continued to produce. Furthermore, the yield of the tomatoes with resistant rootstocks was four times that of the nongrafted susceptible plants. Grafting can be a valuable tool for eliminating bacterial wilt in tomato, pepper, and eggplant production.

Stress tolerance
Grafting has been highly effective at producing plants that can overcome abiotic stressors—the environmental stressors that can lead to decreased yields. More than one-third of all irrigated land worldwide is affected by high salinity. Grafting with salt-tolerant rootstocks can be instrumental in decreasing yield losses. It has also been used to reduce the negative effects of excess moisture in the soil. Grafted plants also have shown effective tolerance to soil temperature extremes, and grafting with certain rootstocks can allow the growing season to be extended in either direction. An extended season can help growers to raise the selling price of their goods as well as add to annual and long-term economic stability.

Increased productivity
The use of grafted vegetables is associated with increased resistance to diseases, abiotic stressors, or both. But grafting can also increase yields without the presence of these stressors. For example, yields increased by as much as 106 percent with the use of certain rootstocks for watermelon production in Australia. Some rootstock varieties have been bred specifically to be used as rootstocks, such as the Maxfiori rootstock used in greenhouse tomato production systems. Use of vigorous rootstock varieties can increase water and nutrient uptake in grafted plants. Many growers worldwide are utilizing these rootstocks to increase fruit yields, even where little disease pressure is evident.

<table>
<thead>
<tr>
<th>Seed Supplier/Rootstock</th>
<th>Disease Resistance</th>
<th>Tomato Mosaic Virus</th>
<th>Fusarium Wilt Race 1</th>
<th>Fusarium Wilt Race 2</th>
<th>Verticillium Wilt Race 1</th>
<th>Verticillium Wilt Race 2</th>
<th>Bacterial Wilt</th>
<th>Nematodes</th>
<th>Vigor</th>
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<tbody>
<tr>
<td>deRutter Seeds</td>
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<td>High</td>
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<td>High</td>
<td>High</td>
<td>Susceptible</td>
<td>High</td>
<td>5</td>
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<tr>
<td>Beaufort</td>
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<td>Moderate</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Susceptible</td>
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<tr>
<td>Takii Seeds</td>
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<tr>
<td>Anchor-T</td>
<td>High</td>
<td>Susceptible</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Moderate</td>
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<td>5</td>
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<tr>
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<td>Susceptible</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Moderate</td>
<td>High</td>
<td>5</td>
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<tr>
<td>Aegis</td>
<td>High</td>
<td>Moderate</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Moderate</td>
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<tr>
<td>Bruinsma Seeds</td>
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<td></td>
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<td></td>
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<tr>
<td>Body</td>
<td>High</td>
<td>High</td>
<td>Susceptible</td>
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<td>High</td>
<td>Susceptible</td>
<td>High</td>
<td>High</td>
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<tr>
<td>Robusta</td>
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<td>Susceptible</td>
<td>High</td>
<td>High</td>
<td>Susceptible</td>
<td>Susceptible</td>
<td>High</td>
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Table 2. Traditional and 2005 international resistance codes for tomato cultivars

<table>
<thead>
<tr>
<th>Scientific Name</th>
<th>Common Name</th>
<th>Traditional Code</th>
<th>2005 International Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato mosaic virus</td>
<td>Tomato mosaic</td>
<td>Tm</td>
<td>ToMV</td>
</tr>
<tr>
<td>Tomato spotted wilt virus</td>
<td>Spotted wilt</td>
<td>TSWV</td>
<td>TSWV</td>
</tr>
<tr>
<td>Ralstonia solanaceae</td>
<td>Bacterial wilt</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Fusarium oxysporum f. lycopersici</td>
<td>Fusarium wilt (Races 0 &amp; 1)</td>
<td>F0 or F2</td>
<td>F0 or 1</td>
</tr>
<tr>
<td>Fusarium oxysporum f. radicicola f. lycopersici</td>
<td>Fusarium crown and root rot</td>
<td>Fr</td>
<td>For</td>
</tr>
<tr>
<td>Pyrenochaeta lycopersici</td>
<td>Corky root rot</td>
<td>K</td>
<td>Pt</td>
</tr>
<tr>
<td>Verticillium dahliae</td>
<td>Verticillium wilt</td>
<td>V</td>
<td>Va</td>
</tr>
<tr>
<td>Verticillium albo-atrum</td>
<td>Vertical wilt</td>
<td>V</td>
<td>Vd</td>
</tr>
<tr>
<td>Meloidogyne spp.</td>
<td>Root-knot nematodes</td>
<td>N</td>
<td>Mj, Mi, Ma</td>
</tr>
</tbody>
</table>

Grafting Step-by-Step

Although grafting is a simple process, it requires careful attention to rootstock selection, seeding dates, healing, and planting in the field. To produce healthy transplants from grafting, follow these steps:

Choose the scion and rootstock cultivars

Any cultivar that provides the desired fruit characteristics can be used to produce scions for grafting. For heirloom tomato growers, popular scion choices include German Johnson, Cherokee Purple, and Kellogg’s Breakfast. Select the rootstock based on its potential for resisting the soilborne diseases on your farm and its ability to enhance marketable yield, even in the absence of disease pressure. Rootstock selection is probably the single most important step in grafting heirloom tomatoes for disease resistance.

To choose the right rootstock, identify the potential pathogens on your farm. Fields where solanaceous crops (tomatoes, tobacco, potatoes, and peppers) have been grown often have recurring problems with bacterial wilt. This disease is widespread in Eastern North Carolina, and many farmers have abandoned fields due to total crop failures from bacterial wilt. In the Appalachian growing regions, vertical wilt causes severe damage to tomato crops because the climate favors the development of this pathogen in the soil. Root-knot nematodes can be diagnosed through soil sampling prior to planting, but occur most frequently in sandy soils. Your county Extension agent can help you learn about basic diagnosis so you can identify potential pathogens in your fields that can hinder productivity.

Many Asian and European companies are releasing varieties that have been bred for rootstock use. Sakata Seed Company and Takii Seeds (Japan) and Bruinsma Seeds and deRuiter Seeds (the Netherlands) have developed lines specifically for rootstock use, but their distribution in the United States may be limited. Table 1 lists some rootstock varieties that are available in the U.S. and their levels of disease resistance.

Although the ideal solution is to find varieties bred specifically for rootstock production, typical hybrids or other modern varieties may also be valuable as disease resistant rootstocks for heirloom cultivars. The key to choosing the right rootstock is to understand how to “break the tomato code.” As breeders have developed genetic lines with resistance, they have designated international resistance codes (Table 2) that can be displayed on labels to identify a cultivar’s resistance to specific diseases. For example, a “Roma Vd Fol 1” variety is one that has been shown to resist race 1 of Verticillium dahliae (Vd) and race 1 of Fusarium oxysporum f. sp. lycopersici (Fol: 1), which are soilborne pathogenic fungi. Many modern hybrids have been bred to resist several pathogens. By using the resistance available in modern tomato breeds, growers may be able to grow some of the tastiest heirloom varieties, even in severely infested fields.

Construct a healing chamber

After the rootstock and scion are grafted together, the resulting plants must reconnect vascular tissue so that water and nutrients can be supplied to the scion. This process occurs in a chamber where humidity, light, and temperature can be regulated. Although construction is relatively simple and inexpensive, proper placement on a farm can be somewhat difficult.

While the grafts are healing in the chamber, they must receive 80 to 95 percent humidity, minimal direct sunlight, and a temperature range between 70 and 80°F. Daily temperature variation must remain low, as additional stress can decrease grafting success. The best place for a healing chamber would be indoors, in a heated storage area or garage, where fluorescent lights can be used during the final days of healing. Healing chambers can also be maintained inside a greenhouse during the spring and fall so long as sufficient shading devices are set up to keep the grafts from being exposed to excessive heat inside the chamber.

A simple healing chamber consists of a frame covered by polyethylene sheeting, which keeps the humidity level high during the healing process. The floor of the
chamber should hold water. During the first days after grafting, an opaque covering is used to keep all light out of the chamber.

At N.C. State University, a number of chambers have been built for tomato grafting. One of the most successful chambers is the simplest and least expensive:

1. Stretch a tarp or dense shade cloth above the greenhouse bench to reduce the full sunlight in the area where the healing chamber will reside. Be sure that the shaded area is much larger than the chamber in order to provide reduced light levels throughout the day and reduce the risk of excessive heat from building up inside the chamber.

2. Place a layer of plastic sheeting on the surface of the bench so that the raised edges of the bench can provide a shallow pool of water on the chamber floor. If a raised lip is not available to help hold water in the chamber, shallow pans of water can be distributed on the bench among the grafts. The use of cool-water vaporizers is an excellent way to increase the humidity within the chamber as long as it does not increase the internal temperature of the chamber.

3. Construct a frame using 1-inch polyvinyl chloride (PVC) piping as illustrated in Figure 2. The frame should have a peak to keep condensation from dripping onto the newly grafted transplants.

4. Cover the PVC frame with a layer of clear plastic so that the sides and ends can be easily pulled up to check on the grafts during healing.

5. Make sure humidity, light, and temperature levels inside the chamber are constant before beginning the healing procedure so that the grafts will be placed into a well-functioning chamber. As noted above, the relative humidity level should be high—between 80 and 95 percent, and the temperature should be a constant 70 to 80°F. Use black plastic to block all available sunlight from entering the chamber until the leaves of the newly grafted transplants attain normal hunger levels—until they no longer show signs of moisture stress.

**Plant the seeds**

When planting the scion and rootstock seeds, use good sanitation practices and a sterile, lightweight potting mix. The grafting process and the subsequent healing time require that seeds be sown two weeks before typical, nongrafted transplant production begins. This allows the newly grafted seedlings to spend up to one week in the chamber followed by one week in the greenhouse to re-acclimate to normal light conditions before they are put into the field.

To achieve a successful graft, make sure that the rootstock and the scion stems are the same diameter. Because different varieties require different germination periods, seeding times may need to be altered to grow different cultivars to the same size. Stagger plantings to offset the effects of variable germination periods between rootstock and scion varieties. In many cases, rootstock varieties take two to five days longer to germinate than heirlooms. However, hybrid rootstock varieties may germinate faster than heirlooms. Plant a few test seeds of each variety (both rootstock and scion) early in the year to determine how long the germination period is for each in your greenhouse or propagation facility. If seedlings have already emerged and either the rootstock or scion is much larger than its corresponding variety, decreasing the environmental temperature can help to slow the growth of the variety that may be ahead of schedule.

**Choose the best time to graft**

Tube grafting should be carried out when the seedlings have two to four true leaves and the stems are 1.5 to 2 millimeters in diameter (Figure 3). For proper healing to
degree of each angular cut does not have to be exact, all cuts should be made at a consistent angle to provide more surface area for the vascular tissue to meet and grow together. Other methods have attempted to increase this surface area even further by making V-shaped cuts, but the results are similar to those of the tube grafting technique. Locate the graft union above the cotyledon to prevent adventitious roots from forming and leading to infection of the susceptible scion tissue (Figure 5).

Once the transplants have been grafted, put them directly into the healing chamber and cover the chamber to prevent any light from reaching the plants.

Monitor the healing process
Immediately after grafting takes place, the plants must form callus tissue and reconnect vascular bundles to provide the scion with nutrients and water. The purpose of the healing chamber is to keep the scion from becoming water stressed during this process. This can be accomplished by slowing the transpiration stream—the movement of water from inside the plant tissue into the atmosphere. The best ways to do this are to increase humidity, decrease light, and decrease temperature. By decreasing the temperature, however, the development of newly forming callus tissue may be hindered. Therefore, the keys to successful chamber operation are high humidity and no direct sunlight while the graft union is developing—between two and four days after making the graft.

The first major constraint to proper graft healing is water stress. Because the scions have been physically separated from their root systems, they tend to wilt, especially in the first few hours after grafting. By adjusting environmental conditions to keep the scions from wilting se-
Figure 6. A typical timeline for grafting. (Rivard and Louws)

Verely, the grafts will have a better chance of survival. Typically, a small amount of wilt during the first day of healing is acceptable. The high humidity level inside the healing chamber will help the scions regain sufficient turgor pressure and show little sign of wilting. Grafts should be left in the chamber for the first two to four days with absolutely no light and high humidity. After these first days, the successful grafts will become apparent as they will regain normal turgor levels while those that were not successful will wilt permanently.

Any movement that pulls the scion stem away from the rootstock will decrease the contact surface between these tissues, ultimately diminishing graft success. Place the grafts into the chamber carefully, inspect the grafts after placing them in the chamber to be sure that the scion has not been pulled away from the rootstock. Excess water on the leaves can also physically pull the scion away from the rootstock. For this reason, do not mist grafts once they are in the chamber. While the grafts are still weak and healing, apply water from the bottom so that the grafts are not subjected to damage from overhead irrigation.

Acclimate the grafts to normal conditions
Throughout the healing process, open the chamber at least twice a day to replenish carbon dioxide for the grafts. After two to four days in the healing chamber with absolutely no light and high humidity, the scions should return to normal turgor levels and display no evidence of moisture stress.

Once the grafts reach normal turgor levels, light can be slowly introduced, and eventually humidity levels can be reduced over the following week. There is a danger that this process can be carried out too fast. When light is first introduced back into the chamber, it is best to simulate indirect light. If the chamber is indoors, mount fluorescent lights above the chamber. In the greenhouse, use a shading apparatus that will significantly reduce the amount of sunlight without eliminating it altogether. As soon as the transplants have

Figure 7. The clip falls after healing.
acclimated to the reduced light level with no signs of stress, gradually decrease humidity by removing pans of water or the cool-water vaporizer and lifting up the sides of the chamber.

Typically, the grafts will require two days at medium light and humidity levels before they can be moved into a low-humidity and high-light environment. Even after the plants have been moved out of the chamber and into a standard greenhouse environment, it is best to water from the bottom to prevent any physical damage to the grafts while the graft union is still weak. Careful overhead watering is possible as long as the seedlings are not exposed to high water pressure that could separate a scion from its rootstock.

As the grafted transplants develop, the grafting clip will expand with the growing stem, and eventually fall off (Figure 7). Once the grafted transplants have been in the greenhouse for five to seven days, they can be managed and planted in the field or greenhouse similarly to nongrafted transplants.

Avoid the pitfalls

Although grafting with resistant rootstock increases the disease resistance of plants in the field, the process may expose transplants to diseases, edema, and disfigurement, all of which could weaken them.

The wound created by cutting the stem provides an easy entry point for bacteria, fungi, and viruses to invade the plant tissue. Therefore, keep the working area, chamber, and all tools extremely clean during this process. Use good sanitation practices to prevent soil contamination, and use lightweight potting mixes that reduce the moisture retention in the soil for proper grafted transplanted production.

Edema, which appears as swollen areas of plant tissue, may form on the leaves if the grafts are allowed to stay in the chamber too long (Figure 8). Edema is a physiological disorder caused by excessive humidity. Plant and monitor the transplants

Figure 9. Transplant so the graft union is well above the soil line.

This is not a serious problem. Move the transplants into an environment with lower relative humidity, and the edema should go away.

If the graft union did not maintain good contact, it may become disfigured. This may cause problems in the field if the union is not strong enough to hold the weight of the vines. Avoid this problem by taking precautions during grafting, planting, and training.

Figure 8. Edema on leaves.

Many tomato growers hope to give transplants an advantage by burying transplants deep into the ground with the stem bent. This practice is not recommended with grafted transplants as susceptible scion tissue may be exposed to soilborne pathogens below ground.

Monitor the grafted tomatoes once they are in the field to keep suckers from robbing the scion of water and nutrients. Suckers are branches that form below the graft union. Often they have a much different morphology than that of the scion. Fruit from the rootstockborne sucker branches will have the genetic makeup of the rootstock rather than the scion. By removing these branches, the scion will be provided with more water and nutrients and increase production of the desired high-quality fruit.

Summary

Grafting is a valuable disease management tactic for heirloom tomato growers. This practice originated as a way to ensure fruit quality while keeping disease resistance high for melon production systems with soilborne disease pressure. This same principle lends itself well to heirloom tomato production. Furthermore, research worldwide has demonstrated increased yields from grafted vegetable plants in comparison to nongrafted plants. As Southeastern growers realize its potential benefits and relative ease, grafting will become a more popular disease management technique in the United States.
Recommended Reading

Acknowledgements
The authors wish to thank Dr. Mary Peet, Dr. Mike Benson, Jim Driver, Bob Stuart, and the N.C. State University Department of Plant Pathology for their assistance with this publication. Cary Rivard provided the cover photograph of a Cherokee Purple heirloom tomato and all other photographs except Figure 8 (by Mary Peet).

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Department of Plant Pathology

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APPENDIX B – EVALUATION OF ‘MAXIFORT’ ROOTSTOCK UNDER CONTINUOUS AND ROTATIONAL FIELD TOMATO PRODUCTION

ABSTRACT

Conventional field production of tomato in the mountain growing regions of North Carolina is limited by the presence of Verticillium wilt, caused by *Verticillium dahliae*. This soilborne pathogen decreases vigor and plant growth, and is particularly problematic due to the lack of rotation common in western North Carolina tomato production. The use of highly vigorous rootstock, ‘Maxifort’, may be a potential alternative method to offset the effects of continuous tomato production. Plant growth was enhanced significantly by the use of ‘Maxifort’ rootstock in rotational and continuous production plots at all sampling dates (P=0.008 and 0.0003). Increases in plant vigor were seen early in the season (35 days after transplanting (DAT)) as a result of rotational management (P=0.003). At the later sampling date (75 DAT), rotational treatments were not significant (P=0.06). ‘Maxifort’ rootstock improved plant vigor compared to its given control within each main (rotational) plot (P=0.0003), but no significant difference was seen among ‘Maxifort’ treatments across the two rotational systems. In contrast, ‘Maxifort’ rootstock under continuous management had similar or better plant vigor than non- and self-grafted treatments in a rotational production system.
INTRODUCTION

Verticillium wilt, caused by *Verticillium dahliae* (race 2), is one of the most important soilborne diseases in the mountain growing regions of North Carolina (Bender and Shoemaker 1984). Endemic populations of this pathogen continue to plague growers as there is no genetic resistance for this race in tomato. Furthermore, increased land-value and agricultural specialization, has led to the abandonment of crop rotation within commercial tomato production. *V. dahliae* leads to poor vigor and fruit set in the field, but aboveground symptoms may not be obvious until late in the season (Pohronezny 1991). Microsclerotia can persist in soil for up to 8 years (Green 1969). However, crop rotation can be carried out (Bhat and Subbarao 1999), and rotational systems may be effective at reducing pathogen severity (Cirulli et al. 1990). Furthermore, continuous cultivation of susceptible host crops increase soilborne inoculum load of this pathogen (Obrien 1983). This suggests that short-term crop rotation may not be effective at eradicating verticillium wilt, but a reduction in severity can occur as a result of crop rotation with non-host species.

Reductions in plant growth even as early as the seedling stage have been seen where *V. dahliae* is present (Baath and Hayman 1983; Karagiannidis et al. 2002). The reduction in plant growth ultimately leads to a poor platform for fruit production and can eventually cause plant death (Bletsos et al. 1997; Cirulli et al. 1990). Many breeders and plant pathologists have attempted to overcome the functional effects of this pathogen by developing lines that are highly vigorous, and can provide adequate nutrients and water before the pathogen overcomes the plant late in the season (Gardner 2006). Grafting with
highly vigorous rootstock lines may be an alternative way of managing the disease by increasing the plant’s growth advantage over the pathogen.

Although the use of grafted vegetables is typically associated with major gene disease resistance and/or abiotic stress tolerance, plant vigor and ultimately yield is often increased even without the presence of these identified stressors. Yield increases have been seen in watermelon production systems (Ruiz and Romero 1999; Yetisir and Sari 2003). For example, in tomato, yield increases in fumigated soil have shown that a vigorous root system can lead to increased productivity (Upstone 1968). Yield increases have been seen in eggplant as well, even without the presence of soilborne pathogens. Grafting eggplant onto wild Solanum rootstock showed that yield increases were significant as compared to self-grafted controls (Ibrahim et al. 2001; Rahman et al. 2002). In greenhouse eggplant production, eggplant grafted onto tomato rootstock showed increased yield as a result of both increased fruit size as well as fruit number as compared to non-grafted controls and those grafted on eggplant rootstock (Passam et al. 2005). Cucumber grafting has also shown similar results as yields were enhanced even without the presence of known soilborne pathogens (Pavlou et al. 2002).

Possible mechanisms for increased yield are probably due to increased water and nutrient uptake among vigorous rootstock genotypes. Stomatal conductance was improved in tomato when grafted onto vigorous rootstock (Fernandez-Garcia et al. 2002). Nutrient uptake for macronutrients such as phosphorus and calcium were enhanced by grafting (Leonardi and Giuffrida 2006; Ruiz et al. 1996). Similarly total N assimilation was increased as indicated by organic N and nitrate reductase activity within the leaf tissue of melon (Ruiz and Romero 1999). Among micronutrients, grafting with certain
rootstocks for melon production augmented Fe uptake as well as the subsequent translocation of this nutrient towards the shoot in relation to the control. Photosynthesis rates were higher in tomato grafted onto wild eggplant genotypes (Matsuzoe et al. 1993).

The purpose of this study was to investigate the potential of grafting with ‘Maxifort’ rootstock to overcome the loss of vigor displayed in tomato grown in fields with a history of *Verticillium dahliae* (race 2). Furthermore, the effect of utilizing crop rotation in grafted and non-grafted production was assessed.
MATERIALS AND METHODS

Grafted and non-grafted transplants were produced at the NCSU Phytotron using the tube grafting technique (Rivard and Louws 2006). NC33EB-1 was used as the scion for grafted treatments and non-grafted controls. NC33EB-1 is a line developed by Dr. Randy Gardner (NCSU Department of Horticulture), and it is well suited for field production in the mountain regions of NC due to the presence of resistance genes for early and late blight.

Field plots were established at the Mountain Horticultural Crops Research Station (Fletcher, NC) and planted on 20 July 2006 within a long-term systems management study. One aspect of the long-term study investigated the effects of rotation for commercial field production of tomato in the mountain regions of NC. Continuous and three-year rotational systems had been implemented for 12 years prior to planting in 2006. The rotational treatment consisted of sweet corn (*Zea mays* L. subsp. *mays*)/fall cabbage (*Brassica oleracea* L.), cucumber (*Cucumis sativus* L.)/fall cabbage, and tomatoes for years 1-6 (two full 3-year rotations) and bell peppers (*Capsicum annuum* L. var. *annuum*); yellow squash (*Cucurbita maxima*)/fall broccoli (*Brassica oleracea* L.); and staked tomatoes for years six through nine, respectively. The continuous tomato treatment was planted in staked fresh market tomatoes every year (same variety as year three in rotation treatment).

A split plot design was implemented whereby main plots consisted of rotation vs. continuous and subplots were composed of grafting treatments. Grafting treatments included non-grafted, self-grafted, and ‘Maxifort’ rootstock. Four replications were planted 20 July and subplots consisted of six plants at 46 cm spacing. Plots were
maintained similarly to typical conventional production practices for the mountain growing region of NC. Raised beds with plasticulture and drip irrigation were used, and plants were trained using the California/Florida stake-and-weave production method.

Plant samples were taken on 24 August and 5 October from the 2nd and 4th plants, respectively for each of the sampling dates (n=4). Shoot biomass was excised by cutting at the graft union and dried at 70 C for 72 hours. Samples were weighed and results were analyzed using ANOVA (PlotIt, Scientific Programming Enterprises, Haslett, MI) and a protected LSD for separation of means was calculated.
RESULTS

On the first sampling date (35 DAT), main plot effects, rotation and grafting, were highly significant and there were no significant interactions between the two (Figure 1A). Continuous tomato production suppressed plant biomass accumulation compared to tomato plant growth observed in rotational plots (P=0.003). Maxifort rootstock enhanced plant growth compared to self-grafted and non-grafted controls (P=0.008). Seventy-five days after transplanting, ‘Maxifort’ impacted plant growth (P=0.0003; Figure 1B). The interaction between rotation system and rootstock was not significant (P=0.05). Analysis of the mean separation indicated that ‘Maxifort’ significantly improved plant growth compared to self-grafted and non-grafted controls within a given rotational management system. ‘Maxifort’ treatments were not significantly impacted across rotational systems. Conversely, self-grafted and non-grafted treatments accumulated significantly less biomass under continuous production. Under continuous management, ‘Maxifort’ had similar or better plant vigor than non- and self-grafted treatments in a rotational production system. This dramatic compensation of ‘Maxifort’ is highlighted by plotting plant growth over time (Figure 2). The plant biomass accumulation of the self-grafted and non-grafted plants is limited between 35 and 75 days after transplanting, whereas biomass accumulation for plants on ‘Maxifort’ rootstock was linear with a strong positive slope. The plant biomass accumulation for ‘Maxifort’ under continuous tomato was even greater than self-grafted plants grown in plots managed with a three-year rotation program (Figure 2).
DISCUSSION:

Crop rotation can affect inoculum potential of soilborne plant pathogens, nutritional status, and general soil health. These factors can play a major role in the productivity of a given cropping system. In this study, the main effects of continuous and rotational field production can be seen by the decrease in plant vigor among non-grafted and self-grafted controls in the continuous plot compared to those in the rotational system (Figure 1A). Furthermore, the rotational effects in the first sampling indicate that rotation is playing a significant role (P = 0.003). The lack of vigor in the continuous production system could be due to the development of Verticillium wilt throughout the field. Crop rotation has been shown to decrease the severity of disease in the field (Cirulli et al. 1990; Obrien 1983). Furthermore, plant vigor can be negatively impacted by *V. dahliae* in tomato as early as the seedling stage (Baath and Hayman 1983; Karagiannidis et al. 2002). At this same sampling point, the use of ‘Maxifort’ rootstock had a significant benefit compared to the other grafting treatments (P=0.008) across both of the main plots.

The ‘Maxifort’ treatment developed enhanced aboveground growth in the continuous treatment compared to that of the self-grafted and non-grafted rotational treatments. Although the accumulation of biomass in the continuous ‘Maxifort’ treatment was not significantly higher than that of the rotational non-grafted treatment, these results suggest that the use of grafting with highly vigorous rootstock may be able to functionally compensate for a lack of rotation in field tomato production. Interestingly, the enhanced growth of the ‘Maxifort’ treatment within the continuous plots resulted in a lack of statistical significance (P=0.06) for this rootstock between main
plots. This further suggests that the use of ‘Maxifort’ in a continuous production system will outweigh the affects of rotation alone.

The use of ‘Maxifort’ rootstock may be advantageous to growers that wish to find alternative ways to manage _Verticillium dahliae_ (race 2) in field and tunnel production. The increased vigor from this rootstock led to a significantly higher aboveground biomass accumulation in the continuous and rotational plots compared to non-grafted and self-grafted controls across both of the rotation treatments. Furthermore, the results from this study indicate that ‘Maxifort’ may be extremely beneficial in a continuous cropping system.

Limitation in space is a major challenge for many growers in this region due to increasing land value. Furthermore, the increased presence and destruction of early blight (caused by _Alternaria solani_ and _Alternaria tomatophylla_) and late blight (caused by _Phytophthora infestans_) has forced many growers into covered structures such as high tunnels or greenhouses. Tunnels and greenhouses provide an excellent cultural control for these foliar pathogens, but it can lead to significant accumulation of inoculum from soilborne pathogens due to lack of rotation or shortened rotational intervals. As growers adopt these innovative growing practices in western NC, grafting technology for vegetable production will be very valuable.
Figure 1 - Shoot biomass of grafted and non-grafted tomato under rotational and continuous production during first (A) and second (B) sampling date. Rotational, grafting, and all treatment interactions displayed (MHCRS, 2006).
Figure 2 - Cumulative shoot biomass production of grafted and non-grafted tomato under rotational and continuous production (MHCRS, 2006).
LITERATURE CITED


Gardner, R.G. 2006. Discussion regarding verticillium wilt in mountain production and grafting. Fletcher, NC.


