ABSTRACT

SILVERMAN, EMILY JEAN. Inoculation Methods and Screening of Selected Tomato Accessions for Bacterial wilt Incidence and Managing Bacterial wilt by Grafting with Disease Resistant Rootstocks in North Carolina. (Under the direction of Frank J. Louws and Dilip Panthee.)

Tomato is an economically important crop worldwide that is plagued by many different plant diseases across all production regions. Bacterial wilt is a destructive disease of tomato caused by Ralstonia solanacearum. Ralstonia solanacearum is a soil-borne pathogen that is widely distributed in sub-tropical and temperate regions attacking over 200 plant species. The wide host range and broad pathogen diversity make bacterial wilt a challenging disease to manage. A multifaceted approach incorporating many management tactics including avoidance, extended crop rotation, cultural practices, sanitation, host resistance, and grafting with disease resistant rootstock. Host resistant is a value tool but unfortunately tomato resistant to bacterial wilt is limited with few genetic resources for breeders to utilize. The objectives of this research were to develop a high throughput screening assay for tomato germplasm advancement and evaluate bacterial wilt resistant rootstocks under field conditions in North Carolina to create grafting recommendations for tomato growers.

Greenhouse experiments were conducted on tomato seedlings to assess the utility of three inoculation methods to find a screening method that produced rapid results, easy to preform, and can be scaled up for high throughput capacity. The soil drench inoculation method was the easiest inoculation method compared to other methods tested and produced the highest mean disease incidence with the least variability of 84.3+/−2.3% compared to leaf snip and dip and root snip and dip inoculation methods, 68.6+/−4.76% and 70+/−2.86%, among seven experiments. The soil drench inoculation method was chosen for screening
germplasm because it was easy to do, gave reproducible rapid results, and was the most similar to natural infection.

The soil drench inoculation method was also used to examine disease response of asexually propagated clones, or cuttings, for a rapid screening assay. A comparison of disease incidence between clones and seedlings subjected to the soil drench inoculation method in experiments above revealed similarities in the rate of disease development with high mean disease incidence of 100+/-21% and 80+/-16% for clones and seedlings, respectively. The initial disease incidence observation at 7 days post inoculation was much higher for clones than seedlings, nevertheless, the disease progress curves showed a near identical slope using simple linear regression. These results suggest seedlings and clones can be used for rapid high throughput bacterial wilt screening.

Thirteen genotypes were screened against two *Ralstonia* isolates (Jackson County and Pender County) in two different environments (a greenhouse and growth chamber) to identify genotypes segregating for resistance to bacterial wilt. Hybrids (4), resistant parents (3), susceptible parents (4), and resistant and susceptible controls (2) showed a range of disease incidence against two *Ralstonia* isolates collected in North Carolina. Among the two most contrasting genotypes in regard to bacterial wilt incidence were CLN1466EA (resistant) and NC84173 (susceptible), parents of NC11212 and subsequent populations of NC11212 were selected for a future gene mapping project based on these results.

Grafting field trials were conducted during the 2012 and 2013 field seasons in severely infested soils in Western North Carolina. In 2012, Cheong gang, BHN1054, RST04-106T, and CRA66 received 25%, 32.5%, 35%, and 43% disease incidence, respectively. In 2013, CRA66, RST-04-106T, Cheong gang, and BHN1054 showed reduce
disease incidence of 2.5%, 5%, 10%, and 27.5%, respectively. The resistant rootstocks demonstrated a yield benefit with higher yields accompanied with lower disease incidence. The non-grafted and self-grafted controls both displayed 100% disease incidence in 2012. The non-grafted and self-grafted controls reached 70% and 63% disease incidence during the 2013 field season, respectively. Grafting with disease resistant rootstocks can help reduce bacterial wilt incidence in severely infested soils and can be a vital tool incorporated into an integrated pest management program to control bacterial wilt.
Inoculation Methods and Screening of Selected Tomato Accessions for Bacterial wilt Incidence and Managing Bacterial wilt by Grafting with Disease Resistant Rootstocks in On-farm Trials in North Carolina

by

Emily Jean Silverman

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APPROVED BY:

______________________________  ________________________________
Frank J. Louws                  Dilip Panthee
Committee Chair

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Asimina Mila                   Peter Balint-Kurti
DEDICATION

To my mother and grandfather who greatly influenced my interest in plant pathology and always encouraged me to follow my dreams.
BIOGRAPHY

Emily Jean Silverman was born in Raleigh, NC on Oct. 30, 1987. Emily grew up in Shotwell, NC on a small farm in a rural setting with boundless opportunities to enjoy nature. Her love of plants came from extended time spent outdoors, hands-on gardening with her mother, and watering plants at the NCDA greenhouse where her mother worked. At the age of 16, Emily was able to work in the Corn Breeding Program led by Dr. Major Goodman, Department of Crop Science, North Carolina State University. Emily’s love for field work came from many long summers working in the corn fields. The corn breeding program was influential in shaping Emily’s career goals. Emily took part in the high school internship program at N. B. Broughton High School and worked with Dr. Goodman and Dr. Jim Holland from NCSU on projects related to the gametophyte factor and mapping of photoperiod response genes in corn. Emily graduated from high school May 2006 and went on to obtain a BS in Horticultural Sciences at NCSU May 2011. Emily took part in independent projects focused on petunia genetics and screening methods development with Dr. Denny Werner and Dr. John Williamson which solidified her interest in plant breeding and plant pathology. In August 2011 Emily started a graduate research assistantship through the Plant Pathology Department at NCSU with Dr. Frank J. Louws.
ACKNOWLEDGMENTS

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CHAPTER ONE

REVIEW OF THE LITERATURE ON BACTERIAL WILT OF TOMATO

SECTION ONE – INTRODUCTION

Fresh-market tomatoes are a large industry in the USA and a large portion of the production occurs in the southeast. Unfortunately, many fresh-market tomato varieties are highly susceptible to a wide array of plant diseases. Some notable plant diseases that cause major issues in tomatoes in the southeast production region include fusarium wilt, verticillium wilt, and bacterial wilt. Bacterial wilt is endemic in the southeast and can cause up to 30% annual crop losses in tomato in North Carolina growers’ fields. Many plant species are susceptible to bacterial wilt including several members of the Solanaceae family (Hayward 1991).

Bacterial wilt is particularly difficult to manage because the bacterium resides in the soil and control relies on many tactics to be effective in reducing disease impact. Management strategies against bacterial wilt include crop rotation, avoidance, host resistance, grafting with resistant rootstocks, cultural control tactics, and sanitation of field equipment. Host resistance and grafting with host resistant rootstocks are sustainable management tactics that are effective and currently under development to improve protection against bacterial wilt. Grafting with resistant rootstocks has been successful and is currently practiced for bacterial wilt management in many tomato production regions around the globe (Black et al. 2003; Lin et al. 2008; Matsuzoe et al. 1993; McAvoy et al. 2012; Peregrine and Bin Ahmad 1982; Rivard and Louws 2008; Rivard et al. 2011; Tikoo 1979). Highly resistant breeding lines derived from diverse tomato germplasm sources have been adapted for
rootstocks and offer protection against bacterial wilt (Rivard and Louws 2008). However, highly resistant breeding lines have not been widely adopted due to poor horticulture traits associated the germplasm. Many new commercial resistant rootstocks are available but have not been evaluated in severely infested soils in North Carolina.
SECTION TWO – BACTERIAL WILT OF TOMATO

*Ralstonia solanacearum*, the causal agent of bacterial wilt, has a wide host range of over 200 botanical species in over 50 plant families including many Solanaceous crops like tomato, tobacco, and potato but can also attack *Musa* sp., *Geranium* sp., and *Zingiber* sp. (Hayward 1991; Kelman 1998). *Ralstonia solanacearum* was first discovered in the 1880’s in tobacco in Granville County North Carolina and described by Erwin F. Smith in 1896 as *Pseudomonas solanacearum* (Kelman 1953). *Ralstonia solanacearum* was first classified as a *Pseudomonas* based on morphological characteristics and later became a member of the *Burkholderia* genus before the most recent designation in the *Ralstonia* genus.

*Ralstonia solanacearum* is a soil inhabitant that survives long periods of time in the soil on crop debris but may also be spread by water movement in the soil or on the soil surface (Louws et al. 2010). The persistence of this soil-borne bacterium in established field production sites has led to large scale crop losses as there are few efficacious management tactics that provide protection under severely infested soil conditions. Extended crop rotation with non-host crops is not economical for fresh-market tomato producers that specialize in the crop. Abandoning agricultural land infested with *Ralstonia solanacearum* is also not economical for most tomato growers. The extensive host range and world-wide distribution of *Ralstonia solanacearum* makes bacterial wilt a difficult disease to manage.
**Ralstonia solanacearum Biology and Epidemiology**

*Ralstonia solanacearum* is a gram negative rod-shaped flagellated bacterium that inhabits the soil. Swimming motility aids the bacterium in movement from plant to plant under saturated soil conditions. *Ralstonia solanacearum* can be spread in irrigation water, as well as through water movement in the soil and on the soil surface, infected plant material, and through contaminated soil or field supplies and equipment (Hayward 1991; Louws et al. 2010). *Ralstonia solanacearum* attacks plants via wound sites and natural openings on roots, then invades cortical tissue, multiplies rapidly within the xylem tissue, and effectively clogs the water conduction system, causing the characteristic wilting symptom (Meng 2013b; Nakaho et al. 2004; Tans-Kerstin et al. 2001). Chemotaxis plays an important role in the host-pathogen interaction of *Ralstonia solanacearum* and tomato. Root exudates attract *Ralstonia solanacearum* to host roots; therefore motility is a very important trait for the pathogen. (Yao and Allen 2006). *Ralstonia solanacearum* produces multiple virulence factors to enable invasion including extracellular polysaccharides, secreted effectors via the type three secretion system, flagella propelled motility via the type four secretion system and cell wall degrading enzymes delivered through the type two section system (Denny 1995; Liu et al. 2005; Meng 2013b; Saile et al. 1997; Tans-Kerstin et al. 2001; van Gijssegem et al. 1995). The extensive host range, world-wide distribution, broad genetic diversity, and multiple virulence factors of *Ralstonia solanacearum* make bacterial wilt difficult to manage.

Solanaceous species are particularly susceptible to bacterial wilt and include crops such as tomato, tobacco, eggplant and potato (Kelman 1998). *Ralstonia solanacearum* can exist in the soil for several years without a host by surviving on crop debris and infected
plant roots which sustain the pathogen before release back into the soil. Well drained soils with high water retention capacity are favorable for \textit{Ralstonia solanacearum} survival (Stall1991).

In tomato, symptoms first appear in new growth and quickly spread to the rest of the plant causing the whole plant to collapse. Adventitious root formation on the stems of infected plants and yellowing of foliage may be observed in some partially resistant cultivars (Stall1991). The presence of the pathogen can be verified by observation of vascular browning of infected plants and isolation of the pathogen. A milky white exudate can be observed streaming from the cut surface of an infected plant stem when placed in water for several minutes; this distinguishes bacterial wilt from other tomato wilt diseases.

\textit{Ralstonia solanacearum} is a highly diverse species complex comprised of four genetically distinct phylotypes that correspond to different geographic origins (Fegan and Prior 2005; Meng 2013a; Prior and Fegan 2005). The phylotypes are classified based on the sequence variation in the highly conserved 16S-26S internal transcribed spacer region and several gene regions related to pathogenicity such as the endoglucanase locus, \textit{hrp}B gene, and \textit{mut}S gene region (Fegan and Prior 2005; Meng 2013a; Prior and Fegan 2005). The phylotypes are subdivided into 23 sequevars that are genetically distinguished by less than 1\% nucleotide variation in the endoglucanase locus (Fegan and Prior 2005; Meng 2013a). Also, clonal lines within a sequevar can be identified and differentiated through genomic fingerprinting (Fegan and Prior 2005). In addition, there appear to be multiple pathotypes such that deployed resistance is frequently not effective against all known strains that share similar characteristics (Chellemi et al. 1994a; Hanson et al. 1996).
Management Tactics

Management of bacterial wilt in tomato field production systems is difficult due to the persistence and pervasiveness of the soil-borne bacterium within infested fields. An integrated pest management program (IPM) is essential to reduce annual bacterial wilt losses. Management tactics that are used to reduce bacterial wilt impact include crop rotation, soil amendments, cultural practices, field equipment disinfection, weed removal, host resistance, and grafting with resistant rootstocks. Crop rotation with non-host crops may reduce the *Ralstonia solanacearum* populations in the soil and subsequently reduce disease incidence. For example, crop rotation with non-hosts such as corn, okra, cowpea, and a partially resistant tomato cultivar significantly reduced bacterial wilt severity and delayed the onset of disease the following season by a few weeks in Nepal (Adhikari and Basnyat 1998). A three to four year crop rotation schedule with non-host crops such as rice, maize, sorghum, carrot, yam, onions, and others can be used to reduce disease incidence (Wang and Lin 2005). In Nigeria, annual crop rotation of tomato with non-host crops like *Mucuna*, *Crotalaria*, and *Cassava* showed reduced bacterial wilt incidence whereas monocropping with native grasses was not effective in protecting the subsequent susceptible crop plantings (Adebayo et al. 2009).

Green manures, crop rotation with non-hosts and intercropping with resistant tomato cultivars has been shown to reduce disease incidence in infested field trials conducted in Nigeria (Adebayo et al. 2009). Cultural practices such as chemical soil amendments, use of biological controls, green manures, and intercropping with non-hosts can improve soil composition while effectively reducing bacterial wilt occurrence in susceptible crops.
(Hartman et al. 1993). In Taiwan, soils amended with calcium oxide and urea showed reduced bacterial wilt incidence in tomato (Michel et al. 1997). It has been reported that nitrogen levels and fertilizer choice can impact the amount of disease; higher nitrogen levels promote disease and conversely, lower nitrogen levels help reduce disease incidence (Kelman 1949). Fertilizer choice can impact bacterial wilt disease occurrence as ammonium-based fertilizers tend to promote *Ralstonia solanacearum* and nitrate forms of fertilizers suppress *Ralstonia solanacearum*. Ultimately, maintaining optimal nitrogen and fertility with soil pH of 6.5-7 can reduce bacterial wilt disease impact (Kelman 1949).

Actigard, acibenzolar-S-methyl, has been used to protect against bacterial wilt. Actigard is effective under low disease incidence and also when coupled with a moderately resistant cultivar but Actigard does not protect susceptible plants in highly infested soil conditions (Pradhanang et al. 2005). Actigard elicits the induction of the systematic acquired resistance (SAR) defense system which provides protection to many plant pathogens. Some biological control options rely on the use of plant growth promoting rhizobacteria (PGPR) which have been shown to reduce disease incidence in infested soils (Gava et al. 2002). *Streptomycyes*, for example, was shown to inhibit *Ralstonia solanacearum in vitro* and reduced incidence in infested soils (Gava et al. 2012).

Proper weed management is also very important because *Ralstonia solanacearum* can survive on weed species that grow in production areas and this inoculum can serve as a reservoir for subsequent susceptible crops (Dittapongpitch and Surat 2003; Moffett and Hayward 1980). Disinfecting field equipment helps to prevent the spread of *Ralstonia solanacearum* especially on wood-based products and packing crates (di Bisceglie et al.)
Tomato growers in North Carolina frequently seek to suppress the pathogen using deep-shanked broad-cast applications of the fumigant chloropicrin in the fall and/or shank injection of chloropicrin within the plastic covered raised beds used for growing fresh market tomatoes. However, fumigation is not effective against Bacterial wilt on tomato (Driver and Louws 2002). Avoidance involves growing tomatoes in production areas without a history of bacterial wilt and not planting in areas that have indigenous populations. Host resistance for bacterial wilt control can be effective, however, often it is strain-specific and resistance does not hold up to all strains tested (Wang et al. 2000). Grafting with host resistant rootstocks is practiced around the world and is effective in reducing bacterial wilt impact in open field production (Black et al. 2003; Lin et al. 2008; McAvoy et al. 2012; Peregrine and Bin Ahmad 1982; Rivard and Louws 2008; Rivard and Louws 2011; Tikoo 1979).

**Grafting with Host Resistant Rootstocks**

Grafting is an ancient art and science that was adapted to herbaceous vegetables in Japan and Korea in the early 1900’s (Lee 2003; Munge et al. 2009). Grafting is the creation of a new plant through fusion of two plants: the rootstock (bottom) and scion (top). Grafting is most commonly used on high-value horticultural crops such as cucurbits and Solanaceous crops. Grafting combines valued traits from a desirable rootstock variety such as vigor, soilborne disease resistance, increased yield, improved fruit quality, and enhanced environmental stress tolerance with a desired fruiting scion variety that meets specific market demands. Grafting has been widely adapted to greenhouse production systems where plants are intensely cultivated to produce high yields on highly vigorous rootstocks (Kubota et al. 2008; Lee 1994; Munge et al. 2009). Grafting methods for vegetable crops including the cleft
graft, tongue graft, and splice/tube graft method (Lee 1994). The splice or tube grafting is the most common approach and this method requires decapitation of both scion and rootstock at a 45° angle. Then the scion top is secured to the rootstock with a silicon tube clip. Tube grafting is used in the commercial production of grafted seedlings and it is highly effective with small plants. High grafting survival rates of 85-98% can be achieved with the use of humidity chambers for healing grafted plants (Oda 1995; Rivard and Louws 2006).

Grafting has been used to reduce bacterial wilt incidence in tomatoes in Asia where disease severity is high due to favorable climate conditions (Peregrine and Bin Ahmad 1982). The breeding line CRA66 provided protection against bacterial wilt under high disease severity in India when used as a rootstock (Tikoo et al. 1979). The breeding line HI7998 also showed high levels of resistance to bacterial wilt when used as a rootstock in severely infested fields in Japan (Oda 1999). The HI7996 and HI7998 lines were both shown to be highly resistant to several Ralstonia solanacearum strains in preliminary inoculation experiments in Taiwan (Lin et al. 2008). CRA66, HI7996 and two hybrid were evaluated and adapted in grafting field experiments to manage Ralstonia solanacearum in North Carolina as well (Rivard et al. 2011). In the case of hybrids that have been evaluated to date, ‘RST-04-105T’ and ‘Dai Honmei’ rootstocks conferred high and intermediate levels of resistance in eastern North Carolina, respectively, whereas the opposite trend was seen in western NC experiments (Rivard et al. 2011). The contrasting performance of diverse resistance rootstocks in different production regions suggests dissimilarities in the environmental conditions and potentially the Ralstonia solanacearum population structure present in different field sites.
SECTION THREE – RESISTANCE TO BACTERIAL WILT IN TOMATO

There are many plant breeding obstacles to overcome in breeding bacterial wilt resistant germplasm. Developing acceptable tomato varieties with resistance is a big challenge because of the complexity of host resistance, pathogen diversity, and environmental conditions present across geographic regions. Host resistance in tomato to bacterial wilt is quantitative and highly influenced by environmental factors (Scott et al. 2005). There are limited sources of bacterial wilt resistance in tomato germplasm and this has led to the development of resistant cultivars that provide incomplete control under high inoculum pressure in diverse geographic regions (Jaworski et al. 1987; Scott et al. 2005). Resistance is strongly influenced by environmental factors with large variation in disease response in cool and warm temperatures (Prior et al. 1996).

Examination of resistant genotypes during disease development demonstrated a physical restriction of bacterial movement into the collar of plant stems during infection. The resistant cultivar, Caraibo showed high tylose formation and this suggested that tylose may be involved in the restriction of bacterial movement through vascular tissue (Grimault et al. 1994). Highly resistant breeding lines, CRA66 and HI7996, showed reduced disease incidence compared to 11 other genotypes and they also demonstrated reduced colonization of collars and mid-stems of plants (Prior et al. 1996). Histological studies of the movement of *Ralstonia solanacearum* through resistant and susceptible genotypes suggest that *Ralstonia solanacearum* colonizes primary xylem tissue but cannot move into the secondary xylem tissue of resistant genotypes. In the highly resistant breeding line HI7996, bacterial
colonization was restricted to the protoxylem and the rest of the primary xylem tissue was not colonized (Nakaho et al. 2000).

**Sources of Resistance to Bacterial wilt**

Sources of bacterial wilt resistance in tomatoes have been identified in *S. lycopersicum* var. *cerasiforme* and *S. pimpinellifolium* (Scott et al. 2005). HI7996 and CRA66 are sources of resistance that provide incomplete control to a broad collection of *Ralstonia* strains but provided protection against specific strains (Jaunet and Wang 1999; Lin et al. 2008). The breeding lines CRA66, HI7996, HI7997, and HI7998 all have demonstrated superior resistance against bacterial wilt in different geographic locations; however, these lines have small fruit size, undesirable vegetative characteristics, and have not been widely adapted by tomato growers (Chellemi et al. 1994a, b; Denoyes et al. 1989; Opena et al. 1990). Tomato resistance to the pathogen is strongly associated with small fruit size which is a big challenge for plant breeders in developing new commercially acceptable resistant cultivars (Abeygunawardena et al. 1963; Denoyes et al. 1989; Opena et al. 1990; Scott et al. 2005). Sources of resistance have been found in related Solanum species such as *Solanum toxicarium*, *S. sisymbriifolium*, and *S. torvum* where resistance was either strain-specific, or broad based, offering resistance to multiple strains in artificial inoculation experiments (Matsuzoe et al. 1993). In Taiwan, eggplant rootstock (EG203) showed the highest level of resistance to a wide range of *Ralstonia solanacearum* strains and eggplant rootstocks are recommended by the Asia Vegetable Research and Development Center (AVRDC) for control of bacterial wilt in tomato production areas with a high risk of flooding (Black et al. 2003). A single dominate resistance gene has been discovered in an eggplant population
derived from resistant sources and demonstrated control under high disease severity conditions (Labeau et al. 2013).

There are many public and private tomato research programs involved in the advancement of bacterial wilt host resistance including but not limited to the AVRDC in Taiwan, Canadian Department of Agriculture, Institut National de la Recherche Agronomique, University of Florida, University of Hawaii, North Carolina State University, BHNSeed, Seminis and other private seed companies (Liedl et al. 2013). There are a few commercially available bacterial wilt resistant tomato varieties including Neptune, Venus, Saturn, Florida 7514, Agatha, BHN669, BHN446, BHN466, Tough Boy 8 F1, Tough Boy 93 F1, and Tropic Boy F1 (Zitter and McGrath 2012; Williams et al. 2010). Neptune is a moderately resistant large frueling cultivar derived from HI7997 and developed in Florida (Scott et al. 1995).

**Screening Tomato Germplasm for Resistance**

Screening germplasm for resistance is a time consuming task and requires multiple artificial inoculations and field evaluations under natural disease severity conditions to fully assess resistance. Accurate bacterial wilt screening is sensitive to the environment and strain used for inoculation (Hayward 1991). Plant density does not have an effect of disease development. In early inoculation experiments, plant densities of 45 to 450 plants were grown in a single flat and demonstrated similar disease incidence results (Winstead and Kelman 1952). In some instances, plant age affected disease development in artificial inoculations and a reduced disease incidence was observed in older plants (Winstead and Kelman 1952; Katawczik and Mila 2012). Larger plants have thicker cortical tissue that may...
provide a barrier in bacterial invasion. Stem rigidity in older plants may be due to an increased production of lignin and younger plants may lack lignin production (Wang et al. 1998; Katawczik and Mila 2012). Asexual clones have also been used for early bacterial wilt screening in an F2 mapping population derived from HI7996 x WVA700 and provided accurate phenotypic response for mapping resistance genes (Thoquet et al 1996). Resistance screening in a controlled environment with seedlings has proven most effective in providing rapid, accurate and reproducible results (Adhikari 1993; Jaunet and Wang 1999; Kelman 1949; Thoquet et al. 1996; Wicker et al. 2007). Seedlings in the five to six week growth stage are ideal for artificial inoculations because symptoms develop quickly and young plants can be used for large scale screening assays with little lag time between evaluations (Winstead and Kelman 1952).

Many inoculation methods have been used to test resistance for bacterial wilt in tomatoes (Adhikari 1993; Kelman 1949; Somodi et al. 1993; Winstead and Kelman 1952). Artificial inoculation methods frequently used include the naturalized soil drench method, stem puncture method, and transplanting into infested soil method (Chellemi et al. 1994a; Katawczik and Mila 2012; Scott et al. 2009; Tans-Kristin et al. 2001). The soil drench method requires wounding plant roots with a sterile scalpel and pouring a concentrated bacterial suspension into the wound sites (Kelman 1949). Many bacterial wilt research programs use the soil drench inoculation method for germplasm screening (Adhikari 1993; Colburn-Clifford et al. 2010; Jaunet and Wang 1999; Somodi et al. 1993; Winstead and Kelman 1952; Xian-Gui et al. 2006). The soil drench inoculation method gave the most rapid results compared to the stem puncture inoculation method in experiments conducted in NC
Later, the soil drench method proved the best for differentiating resistance in greenhouse studies compared to stem puncture, root snip and dip, and transplant to infested soil methods (Winstead and Kelman 1952). The stem puncture method involves placing a drop of inoculum at the intersection of the main stem and a leaf axis nearest the soil and puncturing the stem through the drop of inoculum (Kelman 1949). Transplanting into infested soil can be conducted in the field setting or in a container setting when seedlings are transplanted into containers with inoculated media (Chellemi et al. 1994b; Scott et al. 2009). Other methods such as the root snip and dip and leaf snip and dip methods demonstrated efficacy in screening experiments conducted in a greenhouse and growth chamber setting (Xian-Gui et al. 2006). All artificial inoculation methods require wounding the plant so that the pathogen can gain entry to the xylem for colonization.

Disease response can be evaluated using parameters like disease incidence, area under the disease progress curve (AUDPC), and a bacterial wilt index (BWI). Disease is scored once the plant exhibits wilting symptoms and this is quickly followed by whole plant decline. Disease incidence is scored based on the percent of plants showing wilting symptoms and this is used to create disease progress curves to assess disease development over time. The area under disease progress curve (AUDPC) is a quantitative summary of disease intensity over time and compares average disease intensity between pairs of adjacent time points (Madden et al. 2007). The AUDPC can be calculated with the formula:

\[
\text{AUDPC value} = \frac{\sum (x_i + x_{i+1})}{2} \times (t_{i+1} - t_i)
\]

Where:

\[t_i = \text{time, in days, of each observation}\]
\( x_i = \text{proportion of plants diseased at time, } t_i \)

Bacterial wilt severity is scored based on a subjective rating scale of 0-5 or 0-4; 0=no disease, 1=one leaf wilted, 2=two leaves wilted, 3=whole plant wilted, 4= severely wilted or dead plant, 5=dead plant (McAvoy et al. 2012; Nakaho et al. 2004; Winstead and Kelman 1952). The BWI separates the disease response into severity categories to help differentiate levels of resistance and susceptible response. The plants are scored based on severity of symptoms during screening then the data are used to calculate BWI with the formula:

\[
\text{BWI} = \frac{\sum (\text{severity score} \times \text{number of plants in severity score})}{(\text{highest severity score} \times \text{total number of plants})} \times 100
\]
SECTION FOUR – RESEARCH OBJECTIVES

The research program objectives consist of the following:

1) Conduct greenhouse screening assays that maximize efficiency for bacterial wilt inoculations that can be conducted on young plants, with high throughput capacity, and gives consistently high disease incidence, and rapid results.

2) Use the inoculation method that meets the criteria above to screen multiple F$_2$ populations developed by North Carolina State University for bacterial wilt resistance, including resistant and susceptible parental lines; and select a segregating population for germplasm advancement.

3) To evaluate novel and emerging rootstocks for bacterial wilt resistance under natural disease pressure in different regions of North Carolina.


Hanson, P., and Wang, J.-F. 1996. Variable reaction of tomato lines to bacterial wilt evaluated at several locations in Southeast Asia. HortSci. 31:143-146.


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CHAPTER TWO
INOCULATION METHODS AND SCREENING OF SELECTED TOMATO ACCESSIONS FOR BACTERIAL WILT INCIDENCE

ABSTRACT

Bacterial wilt is a difficult disease to screen against because disease development is highly influenced by environmental factors and resistance to bacterial wilt in tomato is quantitative confounding precise phenotypic observations during evaluations. Three artificial inoculation methods were examined under greenhouse conditions to select an effective screening method for bacterial wilt resistance using the susceptible cultivar Cherokee purple. The soil drench inoculation method provided the highest most consistent disease incidence in seven inoculation experiments with 84.3+/-2.3% mean disease incidence compared to the leaf snip and dip and root snip and dip methods which had 68.6 +/-4.8% and 70 +/-2.9% disease incidence, respectively. The soil drench inoculation method was selected for future screening experiments because it was easy to perform, gave consistent rapid results, and was the most similar to natural infection.

Clones were propagated from mature Cherokee purple tomato plants and inoculated to assess disease incidence and potential use for early bacterial wilt screening for an F2 population mapping project. The clones were collected from mature plants, rooted in Fafard 4P media, and had 100.0% propagation survival. Clones were inoculated with the soil drench method and displayed disease symptoms quickly with 80.0% disease incidence observed at 7 days post-inoculation (DPI) and 100.0% by 28 DPI. Disease incidence of seedlings had a disease incidence at 7 DPI of 50.0%. Simple linear regression of disease incidence data
showed the disease progress curve slopes of clones and seedlings were near identical suggesting similarity in disease development over time. These results demonstrate that clones can be used for rapid disease screening where plant replication is limited due to limited plant material.

Thirteen tomato genotypes were screened against two predominant North Carolina *Ralstonia solanacearum* strains to identify recently bred bacterial wilt resistant tomato F$_2$ populations for a future resistance gene mapping project. Germplasm were inoculated using the soil drench inoculation method in two locations: a greenhouse and a controlled growth chamber. Disease incidence, area under disease progress curves, and a bacterial wilt severity scale 0-4 was used to evaluate germplasm at 7, 14, 21, and 28 DPI. The genotypes with the lowest mean disease incidence from both locations, against two isolates, were HI7998, NC1065, CLN2413A, and CLN1466EA. The hybrid, NC11212, originated from parents that were among the most contrasting with regard to resistance and susceptibility (CLN1466EA and NC84173); population NC11212 was selected for a future mapping project based on results from this screening experiment.
INTRODUCTION

Tomato is an economically important crop in North Carolina making up $34.8 million in sales in 2009 with 112 million lbs. produced across the state (USDA-ERS 2010). North Carolina produced 3.5% of the USA’s total fresh-market tomato production in 2009 on 3,400 acres of production land (USDA-ERS 2010). Tomato production is influenced by many dynamic elements in a variety of geographic regions and plant diseases can be a major determining factor for the amount of yield produced in a specific location. Soil-borne diseases like fusarium wilt, verticillium wilt, and bacterial wilt are ubiquitous in the southeast USA negatively impacting tomato production regions causing large annual crop losses in severely infested soils.

*Ralstonia solanacearum*, the causal agent of bacterial wilt, is a soil-borne bacterium that attacks plants at wound sites on roots, then invades cortical tissue and multiplies rapidly in the xylem tissue building up large populations of bacteria. Once in the xylem, *R. solanacearum* is carried throughout the plant vascular system to effectively prevent the water movement causing the whole plant to wilt (Meng 2013; Nakaho et al. 2004; Tans-Kersten et al. 2001). An integrated pest management program is essential to reduce annual bacterial wilt losses and incorporates tactics such as avoidance, proper site selection, cultural practices, soil amendments, sanitation, host resistance and grafting with disease resistant rootstocks.

Host resistance can be a valuable tool for managing plant diseases but breeding for bacterial wilt resistance in tomato is complicated by many factors. Fresh-market tomato production is largely consumer-driven and growers frequently grow susceptible large fruiting cultivars in spite of large annual crop losses because they can still maintain a small profit.
There is a need for large fruited bacterial wilt resistant cultivars adapted for multiple production regions that protect against a broad range of *Ralstonia* strains to serve growers in meeting market demands while retaining profitability. There is a narrow gene pool of bacterial wilt resistance in tomato germplasm and typically resistance is incomplete to a broad diversity of *R. solanacearum* strains (Chellemi et al. 1994a, 1994b; Hanson and Wang 1996; Scott et al. 2005; Wang et al. 1998). The breeding lines CRA66, HI7996, HI7997, and HI7998 all have displayed excellent resistance to bacterial wilt in different geographic locations but unfortunately these breeding lines have undesirable characteristics linked to resistance and have not been widely adapted by tomato breeders or tomato growers (Chellemi et al. 1994a, 1994b; Denoyes et al. 1989; Opena et al. 1990). Host resistance in tomato to bacterial wilt is quantitative and strongly associated with small fruit size; breaking the linkage of fruit size and resistance remains a big challenge for plant breeders to overcome in developing new resistant cultivars (Abeygunawardena et al. 1963; Denoyes et al. 1989; Opena et al. 1990; Scott et al. 2005). Often when resistance is effective, it is strain-specific providing partial control to endemic *Ralstonia* populations across broad geographic regions (Wang et al. 2000). Bacterial wilt resistant rootstocks RST-04-105T and RST-04-106T have shown a site specific resistance response in North Carolina with preference of RST-04-106T in the mountains and RST-04-105T in the coastal plains region of North Carolina (Rivard et al. 2011). The variable response of these resistant rootstocks to predominate coastal plain and mountain strains suggests variation in host resistance and a diversity of *R. solanacearum* populations may exist in different geographic regions of the state.
Screening germplasm for bacterial wilt is a critical step in advancing resistant genotypes for future line development. Both greenhouse inoculation experiments and field trials are necessary for disease resistance breeding and require enormous amounts of space, time, labor, and resources to conduct and manage properly. Resistance screening in a controlled environment with seedlings has proven effective in providing rapid, accurate, reproducible results (Adhikari 1993; Jaunet et al. 1999; Kelman 1949; Thoquet et al. 1996; Wicker et al. 2007). Cuttings, also called clones, are propagated from mature plants and have been used for early screening in a bacterial wilt mapping project of a segregating F2 population derived from HI7996 X WVA700. Routine asexual propagation was performed on F2 individual stock plants and multiple inoculations were conducted on the clones along with resistant and susceptible parents (Thoquet et al. 1996).

There are several artificial inoculation methods used to screen tomato germplasm for resistance to bacterial wilt (Adhikari 1993; Kelman 1949; Somodi et al. 1993; Winstead and Kelman 1952; Xian-Gui et al. 2006). Three commonly used inoculation methods are the naturalized soil drench method, stem puncture method, and transplanting into infested soil method (Chellemi et al. 1994a; Katawczik and Mila 2012; Scott et al. 2009; Tans-Kersten et al. 2001). The soil drench inoculation method requires wounding plant roots with a sterile scalpel and pouring a large volume of highly concentrated inoculum into the wounded root zone (Winstead and Kelman 1952). The soil drench inoculation method mimics natural infection by introducing *Ralstonia* to the infection court, wounded roots, in the soil where the soil-borne pathogen naturally resides and causes infection. The stem puncture inoculation technique requires placing a drop of inoculum at the junction of the main stem and leaf axis.
near the soil line and puncturing the stem with a needle through the drop of inoculum (Chellemi et al. 1994a; Kelman 1949; Somodi et al. 1993; Winstead and Kelman 1952; Zehr 1970). The stem puncture process is similar to mechanical damage where the main stem is wounded for bacterial entry; however, *R. solanacearum* inhabits the soil and typically infects wounded roots. The third inoculation method commonly used is transplanting healthy seedlings into infested field soils or transplanting seedlings into containers with inoculated soil media under greenhouse conditions (Adhikari 1993; Chellemi et al. 1994a; Scott et al. 2009; Winstead and Kelman 1952). Transplanting healthy seedlings into infested soil in the field or container setting is similar to natural infection by exposing plant roots to the bacteria residing in the soil. Other inoculation methods tested include leaf clipping and submergence into inoculum and root clipping and submergence in bacterial suspension (Xian-Gui et al. 2006). The leaf snip and dip inoculation method requires clipping fresh foliage with sterile scissors and submerging the clipped foliage in a concentrated volume of inoculum for a short period of time before standing the plant upright and allowing excess inoculum to dry on plant surfaces (Xian-Gui et al. 2006). The root snip and dip inoculation method is conducted by washing plant roots clean with sterile water and clipping the terminal root tips then submerging the clipped root tips into concentrated inoculum prior to transplant in fresh sterile media (Xian-Gui et al. 2006).

The tomato breeding program of North Carolina State University is committed to advancing large fruited bacterial wilt resistant cultivars and disease resistant rootstocks to meet tomato growers’ needs and fresh-market tomato demands. The tomato breeding program has developed several F$_2$ populations derived from contrasting bacterial wilt
resistant and large fruited susceptible parents that are suitable for mapping quantitative trait loci (QTL). The hybrids, resistant parents, and susceptible parents of the F2 populations have not been evaluated for bacterial wilt resistance against North Carolina *Ralstonia* strains. These genotypes need to be screened to select a segregating population with the most contrasting parents for QTL mapping of bacterial wilt resistance.

The objectives of the inoculation experiments were: 1) Conduct greenhouse screening assays and compare inoculation methods to identify a technique that maximizes efficiency for bacterial wilt inoculations, 2) use the most efficacious inoculation technique tested to screen multiple F2 populations for bacterial wilt resistance, including resistant and susceptible parental lines; and select a segregating population for germplasm advancement.
MATERIALS AND METHODS

**Ralstonia solanacearum Culture Preparation:**

*Ralstonia solanacearum* cultures were prepared from cryogenic storage and streaked onto casamino peptone dextrose (CPD) basal medium and tetrazolium (TZC) amended medium (French et al. 1995; Kelman 1954). Isolate Rs183, known to be pathogenic on tomato, was used for the inoculation experiments (collected by Dr. Asimina Mila, North Carolina State University, Department of Plant Pathology). Cultures were incubated at 30°C for 48h and single colonies were selected from culture plates based on characteristic milky, fluidal, irregularly shaped colony growth and re-suspended in sterile distilled water and adjusted to $10^8$ cfu/ml concentration (Kelman 1954).

**Inoculation Methods:**

**Leaf Snip and Dip Inoculation Method:** The leaf snip and dip (LSD) inoculations were conducted by clipping the leaflets of the top three leaves of the plant canopy with sterile scissors and dipping the remaining wounded foliage into 30ml of inoculum for 30s then letting excess inoculum drip down the stem once plants were turned upright. Mockinoculated controls were performed by clipping the leaflets of the top three leaves and dipping the plant foliage into 30ml of sterile distilled water (sdH2O) for 30s prior to standing the plant back upright (Xian-Gui, et. al. 2006).

**Soil Drench Method:** The soil drench (SD) inoculation method required wounding plant roots with a sterile scalpel cut 2in deep on either side of the main stem of a plant to create a trench and then 10ml of $10^8$ cfu/ml inoculum as poured into the wounded root zone (Kelman 1949; Winstead and Kelman 1952; Xian-Gui et al. 2006). A second treatment used 20ml of
the same inoculum poured into the wounded root zone. The mock-inoculated SD treatments were wounded with a sterile scalpel and drenched with 10ml sdH$_2$O.

**Root Snip and Dip Inoculation Method:** Plants were uprooted and the root systems were washed prior to the root snip and dip (RSD) inoculations. Roots were cut with sterile scissors removing the terminus 1 to 1.5in of root tips and then immediately dipped into 30ml of inoculum at $10^8$cfu/ml for 30s prior to transplant into fresh moist Fafard 4P media (Winstead and Kelman 1952; Xian-Gui et al. 2006). Mock-inoculated RSD inoculations were conducted by dipping plants into sdH$_2$O for 30s before transplanting to fresh media.

**LSD, RSD and SD Inoculation Experiments:**

The LSD, RSD, and SD inoculation methods described above were assessed in seven separate experiments conducted at different dates under greenhouse conditions (Table 1). The experiments were conducted with greenhouse temperatures ranging from 18.3$^\circ$C+/−2.1 to 36.8$^\circ$C+/−2.0 (Table 1). The cultivar Cherokee purple was sown in Fafard 4P media in cell tray inserts within flats with holes. Seedlings were transplanted post germination into 4in pots containing Fafard 4P media. Seedlings were four to six weeks old for inoculations and the LSD, RSD, and SD inoculation methods were performed as described above with mockinoculated and non-inoculated controls. Observations of wilt and disease incidence (% of plants with symptoms) were scored 7, 14, 21 and 28 days post-inoculation (DPI) until termination of the experiment at 28 DPI. The experiments were set up in a complete randomized design with ten plants inoculated with each method. Each treatment was divided into two replications of five plants each and labeled containers were randomly spaced on a greenhouse bench 4x 20ft.
**Cloning Inoculation Experiment:**

Cherokee purple seeds (84) were sown in cell trays containing Fafard 4P media on May 15, 2012 and seedlings (75) were transplanted to 4in pots in Fafard 4P media 14 days later. Mature plants were fertilized and maintained for several weeks then topped to remove apical dominance and allow for axillary buds to break for clonal propagation. Clones are also known as suckers, cuttings, and propagules which represent a genetically identical replication of an individual. Clones were propagated from mature plants three weeks after they were topped and the clones were rooted into Fafard 4P media in a 72 cell tray insert. The clones were placed under mist and removed 14 days later with 100.0% survival rate. The clones were acclimatized to normal water conditions for an additional 7 days before they were inoculated with the SD method using 10ml of $10^8$ cfu/ml inoculum of isolate, Rs183. Clones were inoculated within the 72 cell tray and placed in a hole-less flat to prevent inoculum escape. The clones were scored for disease incidence (% of plants with symptoms) and observed at 7, 14, 21, and 28 DPI until termination of the experiment at 28 DPI. The experiment was conducted once using propagated clones (72) that were separated into 12 replications of 5 plants each and arranged within the flat. Non-inoculated controls were excluded from the inoculation flat and grown alone in separate six cell inserts on the greenhouse bench 4x 20ft.

**Germplasm Screening Experiment:**

Thirteen genotypes were evaluated in this experiment and included four hybrids used to advance F$_2$ populations (NC1029, NC1065, NC11212, and NC11228) developed by Dr. Dilip Panthee (Table 2). The parental lines with bacterial wilt resistance were developed by the
Asia Vegetable Research and Development Center (AVRDC) including lines CLN1466EA, CLN2413A, and CLN2418A. The susceptible parental lines were developed at North Carolina State University and included NC123S, NC946, NC84173, and 47NC-2 (Table 2). Cherokee purple and HI7998 genotypes were used as susceptible and resistant controls to validate and optimize the screening process.

The pedigrees of the populations and parental lines provide insight to the heritage of bacterial wilt resistance and segregation of resistance genes. The F$_2$ population NC1029_F2 was derived by selfing the F$_1$ hybrid NC1029 developed from a cross of NC123 S (susceptible) X CLN2413A (resistant). The F$_2$ population NC1065_F2 was derived by selfing the F$_1$ hybrid NC1065 developed from a cross of 47NC-2 (susceptible) X CLN2413A (resistant). The F$_2$ population NC11212_F2 was derived by selfing the F$_1$ hybrid NC11212 developed from a cross of NC84173 (susceptible) X CLN1466EA (resistant). The F$_2$ population NC11228_F2 was derived by selfing the F$_1$ hybrid NC11228 developed from a cross of NC946 (susceptible) X CLN1466EA (resistant). The resistant AVRDC breeding line CLN1466EA was derived from a double cross of (CL5915-2-6-2-2-0-4 X CRA84-26-1-3) X (CLN399-19-6-13-12-4 X CRA84-26-1-3). AVRDC line CLN2413A was derived from a double cross of (CLN1617A X CLN1463A) X (CLN1621G X CLN1466D). Breeding line CLN2418A was derived from a double cross of (CLN1621H X CLN1462A) X (CLN1621G X CLN1745B). The susceptible parental line NC123S was derived by selfing the F$_1$ hybrid cultivar ‘Amelia’. Inbred line NC946 was derived by selfing the F$_1$ hybrid developed from a cross of (NC EBR-7 X130S (2002)-1A). The inbred susceptible parental line NC84173 was
derived from a cross of (Fla. 7060 X (NC50-7 X FLMH-1)). Three of the thirteen genotypes do not have well documented pedigree information and include susceptible inbred 47NC-2, Cherokee purple, and resistant breeding line HI7998.

Seeds of each genotype were sown in Fafard 4P media contained in a propagation tray and placed under mist until germinated (4min intervals 4s mist). Seedlings were transplanted to 48 cell trays filled with Fafard 4P media and separated into three distinct treatments: non-inoculated controls, Jackson County isolate inoculated treatment, and Pender County isolate inoculated treatments. Two *R. solanacearum* strains were used for the inoculation experiments to represent diverse geographical regions in Jackson County and Pender County of the mountain and coastal plains of North Carolina, respectively. The experiment was conducted at two separate locations; once in a greenhouse and once in an environmentally controlled growth chamber (Phytotron). Plants were grown under greenhouse conditions (26/22°C day/night temperatures) prior to inoculations in the separate facilities. In the Phytotron, a growth chamber was used to contain the inoculation experiment (30+/−1°C day/night temperature) while the inoculations at Method Road greenhouse was heated continuously with a propagation heating pad (30+/−1°C day/night temperature). All inoculations were conducted using the SD inoculation method with 10ml inoculum as described above. Plants within the three individual inoculation treatments were contained in separate hole-less flats and 6 cell inserts of each genotype were randomly arranged within each separate hole-less flat. Inoculation treatments were maintained separately from one another to ensure no cross-contamination of isolates.
The inoculated plants were observed at 7, 14, 21, and 28 DPI until the experiment was terminated at 28 DPI. Disease incidence (% of plants with symptoms) and bacterial wilt severity scores were assigned weekly. Area under disease progress curve values were calculated based on disease incidence data and were used to assess the amount of disease that occurred over time. Bacterial wilt severity was modeled according to published methods using a 0-4 scale; 0=no disease, 1=one leaf wilted, 2=two leaves wilted, 3=whole plant wilted, 4=dead plant (McAvoy et al. 2012; Nakaho et al. 2004; Winstead and Kelman 1952). These data were then used to calculate a bacterial wilt index (BWI) using the formula:

\[ BWI = \frac{\sum (\text{severity score} \times \text{number of plants within each severity score})}{(\text{highest severity score} \times \text{total number of plants})} \times 100 \]

**Statistical Analyses:**

Statistical analysis was performed with SAS v.9.4 (SAS Institute Inc., Cary, NC). The mixed model procedure (PROC MIXED) was used to analysis disease incidence data and mean separations were calculated using Fisher’s protected LSD test with a significance level of \( P=0.05 \).
RESULTS

LSD, SD, and RSD Inoculation Experiments:

There was no significant difference in disease incidence or AUDPC sums between the LSD, RSD, and SD inoculation methods across seven experiments using the mixed model procedure. The SD inoculation treatment had the highest mean disease incidence of 84.3 +/- 2.3% compared to the LSD and RSD methods which had 68.6 +/- 4.8% and 70 +/- 2.9% disease incidence +/- standard error (Table 1). The AUDPC values showed no statistical significant difference in level of disease between LSD, RSD, and SD inoculation methods (Table 1).

Clone Inoculation Experiment:

Asexually propagated clones from mature greenhouse Cherokee purple tomato plants were inoculated to assess clonal inoculation response for future bacterial wilt screening purposes utilizing the soil drench inoculation method. All clones succumbed to bacterial wilt. The non-inoculated controls did not become infected during the experiment. The initial score 7 DPI was 80.0% and the final disease incidence was 100.0% at 28 DPI (Figure 1A). The clone inoculation disease incidence was compared to the seedlings disease incidence of plants inoculated with the soil drench method experiments (above) to assess the potential of clones for a rapid screening bioassay. Although the experiment was not designed to statistically compare the two methods, the anatomy of the curves is still informative. The clones had a higher initial disease incidence at 7 DPI compared to the seedlings with 80.0% and 50.0% disease incidence, respectively. Clones showed 100.0% disease incidence by 28 DPI whereas seedlings had a mean disease incidence of 84.3% across seven experiments.
Simple linear regression of the untransformed data provided the best fit of disease progress curve data for clones and seedlings with $R^2 = 0.9533$ and $R^2 = 0.9763$, respectively (Figure 1A; Figure 1B). The clone experiment disease progress curve slope was very similar to the slope of seedlings from the SD treatment experiments with slope values of 0.0099 and 0.0154, respectively (Figure 1A; Figure 1B).

**Germplasm Screening Experiment:**

Thirteen genotypes were screened against two *R. solanacearum* isolates using the soil drench inoculation method for the germplasm screening experiment (Table 2). Germination of the thirteen genotypes was poor and resulted in low plant numbers. The experiment was conducted twice in two separate locations in a greenhouse and in a Phytotron growth chamber. The disease incidence, area under disease progress curves, and disease severity data from the greenhouse and the growth chamber were not analyzed statistically due to differences in environmental conditions and variable number of germinated plants and replications in the separate inoculation locations.

The thirteen genotypes showed a range of responses in terminal disease incidence when inoculated with the Jackson County isolate in the Method Rd. greenhouse (Figure 2; Figure 3; Table 2). The least diseased genotype inoculated with the Jackson County isolate was NC1065 with 25.0% terminal disease incidence at 28 DPI (Figure 2; Figure 3; Table 2). The susceptible genotype NC123 S had higher disease incidence than all other genotypes at 7 DPI when inoculated with the Jackson County isolate in the Method Rd. greenhouse (Figure 3). The hybrids ranged in susceptibility to the Jackson County isolate with 25.0%, 67.0%, 75.0%, and 100.0% terminal disease incidence in NC1065, NC1029, NC11212, and
There were also a range of responses in terminal disease incidence of genotypes inoculated with the Pender County isolate at the Method Rd. greenhouse (Figure 2; Table 2). The least infected genotype inoculated with the Pender County isolate was HI7998 with 25.0% disease incidence (Figure 2; Figure 4; Table 2). Two AVRDC lines (CLN2413A and CLN2418A) displayed 50.0% terminal disease incidence when inoculated with the Pender County isolate. The least infected hybrid was NC1029 with 67.0% terminal disease incidence whereas the other three hybrids (NC1065, NC11212, and NC11228) were more susceptible reaching 100.0% disease incidence (Table 2). The most susceptible genotypes inoculated with the Pender County isolate in Method Rd. greenhouse were 47NC-2, NC946, NC84173, NC123S, CLN1466EA, Cherokee purple, NC11228, NC11212, and NC1065 which all reached 100% disease by 28 DPI (Figure 2; Figure 4; Table 2).

There was a range of values in terminal disease incidence among genotypes inoculated with the Jackson County isolate in the Phytotron growth chamber (Figure 2; Table 2). Terminal disease incidence ranged from 40.0% in resistant CLN2413A to 100.0% in susceptible genotypes 47NC-2, NC946, NC84173, NC123S, NC11228, and NC11212 (Table 2). Susceptible genotype NC84173 became infected the fastest and showed higher disease incidence than all other genotypes at 7 DPI when inoculated with the Jackson County isolate (Figure 5). The first four genotypes to become infected at 7 DPI were NC84173, Cherokee purple, 47NC-2, and NC946 (Figure 5). CLN1466EA had less disease at 14 DPI than the other tested genotypes inoculated with the Jackson County isolate (Figure 5). The least diseased genotypes inoculated with the Jackson County isolate at 21 DPI were HI7998,
CLN2413A, and CLN1466EA with 33.0%, 20.0%, and 0% disease incidence, respectively (Figure 5).

Terminal disease incidence ranged from 33.0% to 100.0% among genotypes inoculated with Pender County isolate in the Phytotron. The lowest terminal disease incidence was observed in CLN1466EA and HI7998 with 50.0% and 33.0% disease incidence, respectively (Figure 2; Figure 6; Table 2). The hybrids varied in response to the Pender County isolate with 100.0%, 100.0% 80.0%, and 60.0% terminal disease incidence in NC1029, NC11228, NC11212, and NC1065, respectively (Table 2). Susceptible inbred line NC84173 was more infected than all other genotypes at 7DPI when inoculate with the Pender County isolate (Figure 6). The first five genotypes to become infected were NC84173, NC123 S, NC11228, 47NC-2, and Cherokee purple (Figure 6). The resistant control, HI7998, was less infected than other genotypes at 14 DPI with disease incidence of 0% (Figure 6). HI7998 was also the least infected genotype at 21 DPI with 0% disease incidence when inoculated with the Pender County isolate (Figure 6).

Disease incidence, AUDPC, and BWI data were pooled across two locations (Method Rd. and Phytotron) and against two R. solanacearum isolates (Jackson County and Pender County) in an effort to understand how these germplasm respond across a variety of conditions (Table 2). The lowest terminal disease incidence of 50.0% was observed in the resistant control HI7998 which was considerably lower than all genotypes (Table 2). The hybrids NC1065, NC1029, NC11212, and NC11228 demonstrated variable levels of terminal disease incidence across environments of 61.3%, 73.1%, 88.8%, and 100.0%, respectively (Table 2; Figure 2). The highest total disease incidence was observed in genotypes 47NC-2,
NC84173, NC123 S, NC11228 and Cherokee purple with 100.0%, 100.0%, 100.0%, 100.0%, and 93.8%, respectively (Table 2). The AUDPC sums showed HI7998 was the least infected genotype followed by CLN2413A and NC1065 with 576.0, 958.1, and 984.4 mean AUDPC sums, respectively (Table 2). The hybrids also showed variable disease progress over time with average AUDPC sums of 984.4, 1195.8, 1470.0, and 1618.8 in genotypes NC1065, NC1029, NC11212, and NC11228, respectively (Table 2). The BWI ratings ranged from 97.9 to 32.2 with the lowest BWI observed in genotypes NC1065 and CLN2413A of 42.8 and 32.2 BWI, respectively (Table 2). The hybrids showed variable BWI ratings as well with ratings of 97.9, 93.8, 72.9, and 42.8 in NC11228, NC11212, NC1029, and NC1065, respectively (Table 2).
DISCUSSION

All three LSD, RSD, and SD inoculation methods used in the greenhouse inoculation experiments were able to cause disease with isolate Rs183 in susceptible cultivar Cherokee purple. The most consistent results were seen with the SD inoculation method across all seven experiments based on the standard deviation and standard error of the data. Both the RSD and LSD methods had lower mean disease incidence and produced more variable results; this makes these techniques less useful for accurate disease screening. The SD inoculation method imitated natural infection more closely than the LSD and RSD methods by introducing wounded roots to the soil-borne bacterium in the soil. The SD inoculation method was also the easiest inoculation method to perform. The RSD method required washing roots prior to snipping and dipping for 30s prior to transplanting inoculated seedlings into fresh media. It took several minutes to conduct each RSD inoculation, making the RSD the longest, slowest, most labor intensive inoculation method tested. Although the LSD inoculation method was easy to conduct and required much less inoculum than the SD method, it does not mimic natural infection and gave a more variable disease response. In a similar inoculation experiment, the RSD and LSD methods were the most effective in screening experiments conducted in a greenhouse and growth chamber settings (Xian-Gui et al. 2006). The wounding of roots during artificial inoculation in the SD, RSD, and root snip no dip methods provided good consistent results in Florida inoculation experiments (Somodi et al. 1993).

Plant breeding is a time consuming task and breeding for disease resistance requires screening many progeny early in germplasm development to gain an accurate perspective on
disease response. Disease resistance screening requires accurate phenotyping of many plant replications to verify disease response, however; seedlings in the early stages of inbred line development are required for seed production for future generations of inbreeding and selection. Cuttings or clones could be used as a rapid bioassay for early progeny disease screening in F2 populations derived from single seed decent. A clone represents a biological replication of an individual and can be used in situations when there are limited seed or plant material. Clones produced by cutting have been used for early screening and mapping of bacterial wilt resistance in an F2 population of tomato derived from HI7996 x WVA700 (Thoquet et al. 1996). In our studies, Cherokee purple clones and seedlings from LSD, RSD, and SD experiments inoculated with the soil drench method had a near identical disease progress curve slope and the similarity in disease response curve slopes alludes to the potential use of clones for rapid screening.

The germplasm screening experiment conducted in the environmentally controlled growth chamber in the Phytotron clearly differentiated disease responses of the populations, resistant and susceptible parents, and resistant and susceptible controls. However, the Method Rd. greenhouse location was not as favorable of an environment for differentiation of resistance response probably because of variable environmental conditions. Day length decreased during the experiment at Method Rd. greenhouse in fall 2012 whereas day length was kept consistent in the growth chamber at the Phytotron. The air temperature in the Method Rd. greenhouse was variable compared to the consistent 30 +/- 1 °C air temperature in the growth chamber at the Phytotron. A heating pad was used to promote favorably warm
soil conditions for disease development; however, the high soil temperature was more conducive for high disease incidence than differentiation of resistance.

The variability in disease response of thirteen genotypes in two environments, against two *R. solanacearum*, demonstrates the difficulty in accurate phenotyping of bacterial wilt resistance for specific environments and specific strains. Precise disease assessment relies on large numbers of plant replications to provide reproducible disease results which are essential for disease resistance screening. The preliminary germplasm screening experiment showed the difference between resistant and susceptible genotypes; these results assisted in the selection of population NC11212 for a QTL mapping project of bacterial wilt resistance. The parents of population NC11212 were among the most contrasting genotypes (NC84173, highly susceptible, and CLN1466EA, resistant) and the hybrid showed a range of disease response under different environmental conditions and against two *Ralstonia* isolates. The contrast between resistant and susceptible parents led to the selection of population NC11212 for future research. The recombinant inbred lines derived from population NC11212 will be used to develop large fruited bacterial wilt resistant cultivars adapted for the southeastern USA.
LITERATURE CITED


Hanson, P., and Wang, J.-F. 1996. Variable reaction of tomato lines to bacterial wilt evaluated at several locations in Southeast Asia. HortSci. 31:143-146.


Table 1. Terminal disease incidence, AUDPC sums, and greenhouse temperature ranges for seven LSD, RSD, and SD inoculation experiments conducted using Cherokee purple seedlings over different time. Disease incidence and AUDPC values were analyzed using the mixed model procedure (PROC MIXED). Values of disease incidence and AUDPC sums due to inoculation method are not significantly different from each other (P = 0.05).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Terminal Disease Incidence</th>
<th>AUDPC sums</th>
<th>Greenhouse °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SD</td>
<td>LSD</td>
<td>RSD</td>
</tr>
<tr>
<td>1</td>
<td>80.0</td>
<td>100.0</td>
<td>80.0</td>
</tr>
<tr>
<td>2</td>
<td>80.0</td>
<td>100.0</td>
<td>60.0</td>
</tr>
<tr>
<td>3</td>
<td>90.0</td>
<td>50.0</td>
<td>60.0</td>
</tr>
<tr>
<td>4</td>
<td>90.0</td>
<td>70.0</td>
<td>70.0</td>
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<tr>
<td>5</td>
<td>90.0</td>
<td>50.0</td>
<td>80.0</td>
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<tr>
<td>6</td>
<td>80.0</td>
<td>60.0</td>
<td>70.0</td>
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<tr>
<td>7</td>
<td>80.0</td>
<td>50.0</td>
<td>70.0</td>
</tr>
<tr>
<td>MEAN</td>
<td>84.3</td>
<td>68.6</td>
<td>70.0</td>
</tr>
<tr>
<td>STD DEV</td>
<td>5.4</td>
<td>22.7</td>
<td>8.2</td>
</tr>
</tbody>
</table>
Table 2. Germplasm Screening Chart detailing the genotype, number of seeds screened, seed source, generation, source of resistance, trait description, disease incidence by location (Method Rd. greenhouse and Phytotron growth chamber) and isolate (Jackson County and Pender County), mean disease incidence from both locations (Method Rd. greenhouse and Phytotron growth chamber) and inoculation treatments (Jackson County and Pender County), mean Area under Disease Progress Curve, and mean Bacterial Wilt Index.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of Seeds Screened</th>
<th>Seed Source</th>
<th>Generation</th>
<th>Source of Resistance</th>
<th>Traits Descriptions</th>
<th>Method Jackson</th>
<th>Method Pender</th>
<th>Phytotron Jackson</th>
<th>Phytotron Pender</th>
<th>Mean Disease Incidence</th>
<th>Mean AUDPC</th>
<th>BWI</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI029</td>
<td>12</td>
<td>NCSU</td>
<td>Hybrid</td>
<td>CLN2413A</td>
<td>V1, Fas (1, 2, 3), RNK, TMV, TSWV, large fruit</td>
<td>67.0</td>
<td>67.0</td>
<td>67.0</td>
<td>100.0</td>
<td>75.3</td>
<td>1195.8</td>
<td>72.9</td>
</tr>
<tr>
<td>NCI065</td>
<td>18</td>
<td>NCSU</td>
<td>Hybrid</td>
<td>CLN2413A</td>
<td>BW, Fas 1, GLS, TMV, excellent fruit quality</td>
<td>25.0</td>
<td>100.0</td>
<td>60.0</td>
<td>60.0</td>
<td>61.3</td>
<td>984.4</td>
<td>42.8</td>
</tr>
<tr>
<td>NCI1212</td>
<td>18</td>
<td>NCSU</td>
<td>Hybrid</td>
<td>CLN1466EA</td>
<td>V1, Fas (1 and 2), BW, GLS, large fruit</td>
<td>75.0</td>
<td>100.0</td>
<td>100.0</td>
<td>80.0</td>
<td>88.8</td>
<td>1470.0</td>
<td>93.8</td>
</tr>
<tr>
<td>NCI1228</td>
<td>14</td>
<td>NCSU</td>
<td>Hybrid</td>
<td>CLN1466EA</td>
<td>V1, Fas (1, 2, 3), EB, GLS, BW, TSWV, large fruit</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>1618.8</td>
<td>97.9</td>
</tr>
<tr>
<td>Cherokee purple</td>
<td>14</td>
<td>NC</td>
<td>Inbred</td>
<td>N/A</td>
<td>Excellent fruit quality</td>
<td>100.0</td>
<td>100.0</td>
<td>75.0</td>
<td>100.0</td>
<td>93.8</td>
<td>1553.1</td>
<td>95.8</td>
</tr>
<tr>
<td>CLN1466 EA</td>
<td>6</td>
<td>AVRDC</td>
<td>Inbred</td>
<td>CRA84-26-1-3</td>
<td>BW, Fas 1, GLS, large fruit</td>
<td>100.0</td>
<td>100.0</td>
<td>50.0</td>
<td>50.0</td>
<td>75.0</td>
<td>1137.5</td>
<td>59.4</td>
</tr>
<tr>
<td>CLN2413 A</td>
<td>18</td>
<td>AVRDC</td>
<td>Inbred</td>
<td>CLN1466EA</td>
<td>BW, Fas 1, GLS, TMV, large fruit</td>
<td>75.0</td>
<td>50.0</td>
<td>40.0</td>
<td>80.0</td>
<td>61.3</td>
<td>956.1</td>
<td>32.2</td>
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<tr>
<td>CLN2418 A</td>
<td>18</td>
<td>AVRDC</td>
<td>Inbred</td>
<td>CLN1462A</td>
<td>Heat tolerant, large fruit, BW</td>
<td>100.0</td>
<td>50.0</td>
<td>80.0</td>
<td>100.0</td>
<td>82.5</td>
<td>1137.5</td>
<td>61.3</td>
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<tr>
<td>HI7998</td>
<td>14</td>
<td>Univ. of III</td>
<td>Inbred</td>
<td>PI127805A</td>
<td>Superior BW resistance</td>
<td>75.0</td>
<td>25.0</td>
<td>67.0</td>
<td>33.0</td>
<td>50.0</td>
<td>576.0</td>
<td>56.3</td>
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<td>NCI123S</td>
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<td>Inbred</td>
<td>N/A</td>
<td>V1, Fas (1, 2, 3), RNK, TSWV</td>
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<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>1942.5</td>
<td>93.8</td>
</tr>
<tr>
<td>NCI84173</td>
<td>8</td>
<td>NCSU</td>
<td>Inbred</td>
<td>N/A</td>
<td>V1, Fas (1 and 2), large fruit</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>1925.0</td>
<td>96.9</td>
</tr>
<tr>
<td>NC046</td>
<td>10</td>
<td>NCSU</td>
<td>Inbred</td>
<td>N/A</td>
<td>V1, Fas (1, 2, 3), EB, TSWV</td>
<td>50.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>87.5</td>
<td>1560.4</td>
<td>87.5</td>
</tr>
<tr>
<td>47NC-2</td>
<td>16</td>
<td>NCSU</td>
<td>Unknown</td>
<td>N/A</td>
<td>Excellent fruit quality</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>1785.0</td>
<td>96.7</td>
</tr>
</tbody>
</table>
Table 2 Continued

Q. Number of seeds screened (n) was based on the number of seeds germinated from the total number of seeds sown (26) for each individual genotype.

R. Seeds sources included North Carolina State University (NCSU), Asia Vegetable Research and Development Center (AVRDC), University of Florida (UF), and Totally Tomato (Randolph, WI).

S. Traits are related to germplasm selection and include excellent fruit quality, large fruit, heat tolerance, and resistance to Verticillium race 1 (V1), Fusarium race 1.2, and 3 (Fus), Early Blight (EB), Grey leaf spot (GLS), Bacterial wilt (BW), Tomato spotted wilt virus (TSWV), and Root-knot nematode (RKN).

T. Method Rd. Jackson County Total Disease Incidence was calculated based on the number of plants wilted from each genotype (% disease).

U. Method Rd. Pender County Total Disease Incidence was calculated based on the number of plants wilted from each genotype (% disease).

V. Phytotron Jackson County Total Disease Incidence was calculated based on the number of plants wilted from each genotype (% disease).

W. Phytotron Pender County Total Disease Incidence was calculated based on the number of plants wilted from each genotype (% disease).

X. Mean disease incidence was calculated based on the disease incidence across two locations and against two *R. solanacearum* isolates for each genotype.

Y. AUDPC sums were calculated based on the cumulative disease incidence over time for each genotype across two locations and screened against two *R. solanacearum* isolates.

Z. Bacterial wilt index (BWI) was calculated based on a disease severity scale of 0-4 with 0= no symptoms, 1=one leaf wilt symptoms, 2=two leaf wilt symptoms, 3=three leaves or whole plant wilt symptoms, 4=severely wilted or dead; [Σ (score X number of plants/score)/ (total number of plants X highest score)] x 100. BWI was calculated based on disease severity of for each genotype across two locations and against two *R. solanacearum* isolates.
Figure 1 A and Figure 1 B. A. Clones were propagated from mature Cherokee purple greenhouse tomato plants and subjected to the soil drench inoculation method to assess clone response for rapid bacterial wilt screening. Each data point represents the mean disease incidence from 12 replications of the clonal inoculation experiment. Simple linear regression was performed on untransformed disease incidence to visualize linear disease progress for comparison. B. Soil drench inoculated Cherokee purple seedlings from the seven LSD, RSD, and SD experiments were compared to clones subjected to the soil drench inoculation method to assess the differences in disease response. Each data point represents the mean disease incidence from 14 replications from SD means of the seven SD experiments. Simple linear regression was performed on untransformed percent disease incidence to visualize linear disease progress for comparison.
Figure 2. Germplasm Screening Experiment Total Disease Incidence from two growth facilities (Method Rd. greenhouse and Phytotron growth chamber) and inoculated with two separate *R. solanacearum* isolates (Jackson and Pender County isolates).
Figure 3. Method Rd. greenhouse disease progress curves of thirteen genotypes inoculated with the Jackson County *R. solanacearum* isolate using the soil drench inoculation method. Inoculation treatments were supplied supplemental heat through continuous heating from a propagation heating pad (30°C+/−1 °C).
Figure 4. Method Rd. greenhouse disease progress curves of thirteen genotypes inoculated with the Pender County *R. solanacearum* isolate using the soil drench inoculation method. Inoculation treatments were supplied supplemental heat through continuous heating from a propagation heating pad (30°C+/−1°C).
Figure 5. Phytotron disease progress curves of thirteen genotypes inoculated with the Jackson County *R. solanacearum* isolate using the soil drench inoculation method. Inoculation treatments were container with a controlled environment growth chamber (30°C+/−1°C).
Figure 6. Phytotron disease progress curves of thirteen genotypes inoculated with the Pender County R. solanacearum isolate using the soil drench inoculation method. Inoculation treatments were container with a controlled environment growth chamber (30°C+/−1°C).
CHAPTER THREE
MANAGING BACTERIAL WILT OF TOMATOES BY GRAFTING WITH DISEASE RESISTANCE ROOTSTOCKS IN NORTH CAROLINA

ABSTRACT

*Ralstonia solanacearum* is the causal agent of bacterial wilt of tomatoes. *Ralstonia solanacearum* infects many members of the *Solanaceae* family including eggplant, potato, and tobacco. Grafting with disease resistant rootstocks has proven effective in reducing bacterial wilt impact in tomato, but rootstocks are not durably resistant in all regions where the pathogen is present due to pathogen diversity or due to changes in the pathogen population that overcomes deployed resistance. Therefore it is critical to assess tomato rootstocks from a range of sources to determine effective rootstocks for North Carolina growers to implement grafting in bacterial wilt management programs.

On-farm grafting experiments were conducted in western North Carolina to test the efficacy of grafting for bacterial wilt management in 2012 and 2013. The most resistant rootstocks from the 2012 field trial were Cheong gang, BHN1054, RST-04-106T, CRA66, and BHN998 with 25.0%, 32.5%, 35.0%, 42.75%, and 45% terminal disease incidence, respectively. Rootstock RST-04-105T (DP Seeds), non-grafted, and self-grafted controls proved to be highly susceptible to the *Ralstonia* strains present in the Jackson County field site displaying 100% disease incidence by 74 DPI. Bacterial wilt resistant rootstocks HI7998, BHN998, and BHN1053 demonstrated moderate levels of resistant with 45%, 53%, 60%, and 69% disease incidence, respectively and the non-grafted and self-grafted treatments had 100% incidence.
In 2013, the least infected rootstocks were CRA66, RST-04-106T, HI7997, Cheong

gang, and HI7998 with 2.5%, 5.0%, 7.5%, 10%, and 10% disease incidence, respectively.
Non-grafted and self-grafted controls reached 70% and 63% disease incidence, respectively,
during the 2013 field season. Rootstocks RST-04-105T and Maxifort proved susceptible with
disease incidence of 65% and 77.5%, respectively. Grafting is a sustainable management tool
that can be used to reduce bacterial wilt impact of tomatoes grown on infested field sites.
INTRODUCTION

*Ralstonia solanacearum* is the causal agent of bacterial wilt of tomatoes. The soilborne bacterium inhabits soils of tropical and temperate regions around the world, including the southeastern USA. *R. solanacearum* causes large annual crop losses within the *Solanaceae* family including economically important crops such as tomato, eggplant, potato, and tobacco (Hayward 1991). In the case of tomato, bacterial wilt is a devastating disease characterized by the rapid wilting of new foliage at the growing points, quickly followed by total plant decline. The motile soil-borne bacterium enters plant roots and gains entry to the xylem tissue where bacteria proceed to clog the water column. Once bacteria are in the xylem, through quorum sensing, they produce extracellular polysaccharides and cell wall degrading enzymes that ultimately cause xylem clogging and tissue maceration (Denny 1995; Salie et al. 1997). Bacterial wilt occurs across the state, from the sub-tropical eastern coastal plain to the temperate western mountain production regions of North Carolina.

Management of bacterial wilt in tomato field production systems is difficult due to the persistence and pervasiveness of the soil-borne bacterium within infested fields. Integrated management tactics focus on avoidance, sanitation, crop rotation, cultural practices, fumigation, host resistance, and grafting with disease resistant rootstocks. Host resistance and grafting with bacterial wilt resistant rootstocks has proven effective in reducing disease incidence in the southeast USA and other parts of the world (Rivard et al. 2011; McAvoy et al. 2012; Peregrine and Bin Ahmad 1982).

Grafting is an important horticultural technique that is widely used in the production of perennial and herbaceous fruits and vegetables. Grafting herbaceous fruits and vegetables
was commercially adapted in Japan and Korea in the early 1900’s with Cucurbits (Lee 2003; Munge et al. 2009). Grafting has been extensively used in the greenhouse production of horticultural crops where plants are intensely cultivated to produce high yields on highly vigorous rootstocks (Kubota et al. 2008; Lee 1994; Munge et al. 2009). Grafting combines valued traits from a desirable rootstock variety such as enhanced vigor, soil-borne disease resistance, increased yield, improved fruit quality, and environmental stress tolerance with a desired fruiting scion (grafted top) variety. Grafting enables the deployment of diverse sources of rootstocks to manage site-specific issues while growing market-preferred fruit cultivars as the scion.

Grafting has been used to reduce bacterial wilt incidence in tomatoes in Asia where disease severity is high due to favorable climate conditions (Peregrine and Bin Ahmad 1982). The breeding line CRA66 displayed low disease incidence of bacterial wilt under high disease severity in India when used as a rootstock (Tikoo et al. 1979). The HI7998 breeding line also showed high levels of bacterial wilt resistance when used as a rootstock in severely infested fields in Japan (Oda 1999). The HI7996 and HI7998 lines were both shown to be highly resistant to several *R. solanacearum* strains in inoculation experiments conducted in Taiwan (Lin et al 2008). CRA66, HI7996 and two hybrid lines were adapted and evaluated in grafting field experiments to manage *R. solanacearum* in North Carolina as well (Rivard et al. 2011). In the case of hybrids evaluated to date, ‘RST-04-105T’ and ‘Dai Honmei’ rootstocks conferred high and intermediate resistance levels in eastern North Carolina, respectively, whereas the opposite trend was seen in western North Carolina experiments with ‘RST-04-105T and ‘Dai Honmei’ showing low resistance or no resistance (Rivard et al. 2011).
Given the devastating losses bacterial wilt can cause and lack of effective rootstocks that currently can be recommended, there is a critical need to evaluate more commercially available rootstocks in infested fields to develop rootstock recommendations for the North Carolina tomato industry. This research was undertaken to evaluate novel and emerging commercially available rootstocks for bacterial wilt resistance under natural disease pressure in North Carolina.
MATERIALS AND METHODS

The susceptible cultivar Florida 47 (Harris Seeds) was used as a scion in grafted trials conducted in Jackson County as preferred by the cooperator. Rootstock treatments evaluated in 2012 included Non-grafted, Self-grafted, CRA66 (UFL-Dr. Jay Scott), HI7998 (UFL-Dr. Jay Scott), BHN998 (BHN Seed, Inc), BHN1053 (BHN Seed, Inc), BHN1054 (BHN Seed, Inc), RST-04-105T (DP Seeds), RST-04-106T (DP Seeds), Shincheong gang (Seminis Vegetable Seeds, Inc), and Cheong gang (Seminis Vegetable Seeds, Inc). Rootstock treatments evaluated in 2013 included Non-grafted and Self-grafted Florida 47, CRA66, HI7997 (UFL-Dr. Jay Scott), HI7998, BHN1053, BHN1054, RST-04-105T, RST-04-106T, Maxifort (DeRuiter), and Cheong gang.

Scion and rootstock seeds were sown in Fafard 4P soil media in 72 cell trays. Seeds were sown based on the number of days required before grafting, as determined by a preliminary seed germination assay (Table 1). Germination, emergence of the hypocotyl from the media, was recorded for all trials between 8 to 18 days post-seeding (DPS). Seedlings were grown for 18 to 28 days to the 2mm stem diameter size which corresponded to the 3 or 4 leaf stage and is optimum for grafting (Rivard and Louws 2006). The splice grafting method was used for all trials (Rivard and Louws 2006). Briefly, the plants were acclimated to indoor grafting location conditions several hours prior to grafting. A sterile scalpel was used to decapitate rootstock seedlings at a 45° angle below the cotyledons and the scion plants were decapitated above the cotyledons at a 45° angle, and saved for grafting. A clear silicone clip was placed at the cut edge of the rootstock stem and the scion top was
secured within the silicone clip, matching the angle of the cut. Then, plants were placed in a healing chamber under low light and high humidity conditions.

A healing chamber was constructed for the 2012 grafting experiments in the Phytotron at NC State University within a controlled environment growth chamber at 26/22°C +/- 0.5°C (12hr photoperiod, 8ft VHO fluorescent lamps, and standard 100Watt incandescent lighting). The healing chamber design was modeled after previous work (Rivard and Louws 2006) with a large external frame that was wrapped with plastic sheeting, draped with multiple layers of shade cloth, and had a humidifier (Trion 707u Herrmidifier) to direct humidity into the enclosed healing chamber. A modified healing chamber was constructed in 2013 in a shaded, cool greenhouse unit with an existing overhead mist system in the Mary Ann Fox Greenhouse. The second healing chamber was assembled by draping the external mist bench frame with multiple layers of shade cloth in a 3.05m x 1.2m bench area. The mist bench was automated to apply mist at 8 min intervals with 4 s mist durations for a 12 hour period during daylight hours (CoolNet Pro, cross four nozzle configuration, 65 micron average droplet size, output ~7.5-14mL/nozzle unit/mist event, Netafim). The overhead mist system nozzles were suspended 45cm above grafted plants, spaced 45cm apart, and attached to PVC piping from the topmost support of the external bench frame.

Grafted plants healed over a 7 to 10 day period by gradually removing layers of shade cloth from the chamber day by day then reducing the mist conditions over several days to acclimate plants to standard greenhouse conditions. Mist frequency was decreased during the healing process to reduce water droplet formation and droplet accumulation on leaf surfaces when observed. Additionally, mist frequency was increased to 4min intervals with 4s mist
to reduce drought stress as needed. Grafted plants were hardened off in greenhouse conditions for 7 days prior to transplant. Grafting survival was recorded prior to transplant.

Two on-farm trials were conducted in a field location with a history of Bacterial wilt during the 2012 and 2013 field seasons. The Jackson County trials were conducted in the mountains, on a commercial farm in Whittier, North Carolina (35.420844, -83.340680) with Braddock clay loam (pH=6.5). Plants were transplanted with 46cm in row and 1.5m between-row spacing (Rivard et al. 2010). On-farm trials were transplanted on June 25th, 2012 and June 27th, 2013 and the trials lasted for 92 and 90 days after transplant (DAT), respectively.

A Randomized Complete Block Design with 4 replications was used for all field trials. Twelve-plant plots were desired for the 2012 trials but lower numbers of plants were planted for specific treatments (5-12 plants/plot) due to inconsistent grafting survival rates. All data were normalized according to the plant stand in each plot. All plots had 10 plants/plot in 2013.

Plants were transplanted at the soil line of the seedling cells which corresponded to the grafting union remaining 2.5-3cm above the soil line. A standard stake and weave system was used to manage plants. Plants were pruned below the graft union to prevent rootstock sucker growth and were suckered up to the third axil from the graft union. Fertilizer and pest management practices were performed using standard recommendations for commercial production (Ivors et al. 2010).

Observations were recorded on a weekly to biweekly basis. Data collected included disease incidence, plant height, plot vigor and yield. Bacterial wilt disease incidence was
scored based on the number of plants in each plot that exhibited wilt symptoms caused by *R. solanacearum*. Destructive plant samples were collected after plants were scored as dead to verify the pathogen and cause of death. Plant height (cm) was collected on a weekly basis after 14 DAT by measuring from the base of the plant at the soil line up to the growing point of the main stem. Plot vigor ratings were assigned to each plot based on a subjective rating scale from 1 to 10, with 1 representing a poor looking plot with mostly dead plants and a rating of 10 indicating healthy, good looking plots. Plots were harvested as fruit matured and yield was recorded and sorted based on marketable quality. A once over harvest was conducted in 2012 due to high disease incidence, and in 2013 the plots were harvested 4 times.

All data were analyzed with SAS v9.4 (SAS Institute, Cary, NC) with Enterprise Guide 6.1 and JMP v11 (SAS Institute, Cary, NC). Data were analyzed using the mixed model procedure (PROC MIXED) and mean differences in rootstock effects were separated with Fisher’s protected LSD test using a significance level of *P*=0.05. Disease progress curves were created based on disease incidence and area under the disease progress curve (AUDPC) values were calculated and analyzed using the mixed model procedure. Total yield (marketable and cull weights), marketable yield, and marketable fruit count were analyzed using the mixed model procedure and calculated to represent tons/hectare (t/ha) in the figures. Plant height and plot vigor were analyzed using the mixed model procedure.
RESULTS

High disease severity was present in 2012 in the field site with non-grafted and self-grafted Florida 47 controls reaching 100% disease incidence by 74 DAT (Figure 1). The RST-04-105T rootstock treatment also performed poorly developing 100% disease incidence by 75 DAT. The presence of *R. solanacearum* was confirmed based on diagnostic symptoms, bacterial streaming from wilted plants, positive and negative ELISA assays, and isolations of pure cultures using semi-selective media.

In contrast, the Cheong gang rootstock treatment had the least number of plants infected, with 25% disease incidence. The area under the disease progress curves (AUDPC) highlight a highly resistant group of rootstocks comprising Cheong gang, BHN1054, CRA66, RST-04-106T, and Shincheong Gang and a moderately resistant group comprising, BHN998, HI7998 and BHN1053 (P<0.0001; Figure 2).

Rootstock selection had a significant impact on total marketable yields (P=0.0007; Figure 3). The non-grafted and self-grafted treatments produced 1.61 t/ha and 0.95 t/ha marketable yield, respectively, and were comparable to HI7998, BHN998, BHN1053, RST04-105T, and Shincheong gang marketable yields of 29.9 t/ha, 34.2 t/ha, 20.4 t/ha, 8.8 t/ha, and 34.0 t/ha, respectively (Figure 3). The four rootstock treatments that produced the highest marketable yields include Cheong gang, CRA66, BHN1054 and RST-04-106T, and with 74.4 t/ha, 41.4 t/ha, 41.0 t/ha and 36.6 t/ha, respectively. Total yields were also significantly different among rootstock treatments with Cheong gang producing significantly higher yields than all other rootstock treatments (P<0.0001; Figure 4).
Plant height was not significantly different among rootstock treatments and ranged from 82.8 cm in Cheong gang, the least diseased rootstock, to 41.4 cm in RST-04-105T, one of the most susceptible rootstocks. Terminal plot vigor ratings were significantly different among rootstocks and vigor was a valuable parameter in the evaluations of rootstock performance (P<0.0001; Figure 5). The mean separations showed the division of the most vigorous rootstocks (Shincheong gang, BHN1054, RST-04-106T and BHN998) compared to the moderately vigorous rootstocks (Cheong gang, CRA66 and BHN1053), least vigorous rootstocks (HI7998, RST-04-105T) and the controls (Self-grafted, and Non-grafted).

Bacterial wilt incidence in 2013 in Jackson Co. was high in the susceptible controls and rootstock effects were dramatic (Figure 6-7). Bacterial wilt was verified based on diagnostic symptoms, bacterial streaming from infected plants, ELISA assays, and isolation of pure cultures. The most susceptible treatments included Maxifort, Non-grafted control, RST-04-105T, and Self-grafted control with 77%, 70%, 65%, and 63% disease incidence (Figure 6).

The CRA66 rootstock had the least number of plant infected with only 2.5% disease incidence even after 77 DAT. Rootstocks RST-04-106T, HI7997, Cheong gang, and HI7998 also showed high levels of resistance with 5%, 7.5%, 10%, and 10% disease incidence, respectively. Rootstock treatments BHN1054 and BHN1053 offered modest control of bacterial wilt displaying 27.5% and 37.5% incidence by the 77 DAT (Figure 6). Non-grafted and self-grafted controls proved susceptible with 70% and 63% disease incidence, respectively. Rootstocks RST-04-105T and Maxifort had were also susceptible to the Ralstonia strain present displaying 65% and 77.5% disease incidence by the end of the field trial. The AUDPC values highlight a highly resistant group of rootstocks that included
CRA66, RST-04-106T, HI7997, Cheong Gang, HI7998 and BHN1054 (P<0.0001 Figure 7).

There was a significant impact of rootstock selection on marketable yield (P<0.0001; Figure 8). The four leading rootstock treatments include Cheong gang, RST-04-106T, CRA66 and BHN1054 with 63.5 t/ha, 59.3 t/ha, 54.9 t/ha, and 45.9 t/ha total marketable fruit, respectively. Rootstock treatments BHN1053, HI7997, self-grafted, and HI7998 produced adequate yields under infested soil conditions with 36.9 t/ha, 23.8 t/ha, 23 t/ha, 22.6 t/ha, and 45.9 t/ha total marketable fruit, respectively. The treatment with the lowest marketable yield was RST-04-105T with 5.9 t/ha. Total yield (marketable weight plus cull weight) was also impacted by rootstock treatments (P<0.0001; Figure 9). Cheong gang, RST04-106T, and CRA66 treatments produced the highest total yields of 64.2 t/ha, 60.6 t/ha, and 58.7 t/ha which was significantly higher than all other treatments (Figure 9). Moderately infected rootstocks BHN1054, BHN1053, self-grafted, HI7997, HI7998, non-grafted, and Maxifort produced comparable total yields with 47.55 t/ha, 38.8 t/ha, 26.35 t/ha, 26 t/ha, 24.65 t/ha, 20 t/ha, and 16.43 t/ha, respectively. The lowest yielding rootstock treatment was RST-04-105T with 8.24 t/ha total yield produced (Figure 9).

Plant heights were not statistically significantly different among rootstocks and ranged from 66.3 cm in the non-grafted control to 48.3 cm in HI7997 rootstock plots. Terminal plot vigor ratings were statistically significantly different among rootstocks (P<0.0001; Figure 10). The most vigorous rootstock was CRA66 followed by Maxifort and RST-04-106T with mean terminal ratings of 5.5, 5.5, and 5.3, respectively. The least vigorous rootstocks included the self-grafted treatment, HI7998, RST-04-105T, and the non-grafted control with 2.50, 2.25, 2.00, and 2.00, respectively. BHN1054, Cheong gang, HI7998, and
BHN1053 were moderately vigorous rootstocks in the presence of disease with terminal vigor ratings of 4.75, 4.75, 4.25, and 3.75, respectively (Figure 10).
DISCUSSION

Grafting with disease resistant rootstocks in naturally infested field locations is a sustainable agriculture practice that effectively reduces disease incidence and has the potential for yield and plant vigor benefit (McAvoy et al. 2012; Rivard et al. 2008). Grafting for bacterial wilt control in tomatoes is practiced around the world with many different sources of resistant rootstocks (Grimault and Prior 1994; Lin et al. 2008; Matsuzoe et al. 1993; McAvoy et al. 2012; Peregrine and Bin Ahmad 1982; Rivard and Louws 2008; Rivard et al. 2011; Tikoo et al. 1979). Grafting susceptible fruiting cultivars onto resistant rootstocks is effective in reducing disease while maintaining the desired fruit for fresh-market consumption. However, rootstocks are frequently effective in region of the world and not in others, and there may be potential that rootstocks may fail if constantly used in the same field due to changes or selection in the pathogen population.

This study demonstrated Cheong gang, RST-04-106T, and BHN1054, commercially available hybrid rootstocks, provided superior control of bacterial wilt in western North Carolina. Higher survival rates conferred by these hybrid selections resulted in dramatic yield improvements compared to non-grafted and self-grafted controls in the presence of pathogen inoculum. Despite high disease incidence, moderately resistant rootstocks and susceptible grafted treatments produced comparable marketable yields to the highly resistant rootstocks.

The open-pollinated breeding line CRA66 was also found to have superior resistance to *R. solanacearum* strains present in western North Carolina and produced a yield benefit comparable to the hybrids. This breeding line has potential for use by organic and small
acreage growers with bacterial wilt infested soils seeking sustainable agriculture alternatives for disease management and who may elect to grow their own seed. The other breeding lines such as HI7998 and HI7997, conferred good resistance against the pathogen but this did not translate into superior yields and are therefore not recommended for use in commercial settings.

Cheong gang was also evaluated in Virginia in 2010 and found to be highly resistant with only 6.5% disease incidence and conferred acceptable yields of 78.9 t/ha (McAvoy et al. 2012). Likewise, in Florida Cheong gang had 0% and 28.4% disease incidence and produced 52.2 t/ha yield and 28.7 t/ha total yield in the spring and fall of 2010, respectively (McAvoy et al. 2012). Therefore Cheong gang appears to have broad regional use as a rootstock to reduce bacterial wilt incidence.

Rootstocks RST-04-105T and RST-04-106T have been used in grafting evaluations across the southeast USA with variable resistance in diverse geographic regions. RST-04105T was previously reported in Florida to have moderate resistance with 47.6% disease incidence while RST-04-106T had 19% disease incidence (McAvoy et al. 2012). In Virginia, RST-04-105T proved more susceptible than RST-04-106T with 11.2% incidence compared to 1.5% incidence, respectively (McAvoy et al. 2012). Higher disease incidence was noted in Jackson Country NC grafting trials conducted in 2009 with Mountain Fresh grafted onto RST-04-105T than with Mountain Fresh grafted onto Dia Honmei, with 21% disease incidence compared to 0%, respectively (Rivard et al. 2011). Conversely, in Sampson County, NC 2007 grafting trials with Celebrity grafted onto RST-04-105T rootstock showed no wilt symptoms while the Dia Honmei treatment had 50% disease incidence. In 2009 in
Henderson County, NC rootstock RST-04-105T also performed poorly with 65% disease incidence (Rivard et al. 2011). In this study, RST-04-105T had 100% disease incidence in 2012 Jackson County trials and in 2013 under lower disease pressure, RST-04-105T had 65% disease incidence; more than all the other bacterial wilt resistant rootstock selections. RST04-106T in the same location exhibited 35% and 5% disease incidence in 2012 and 2013, respectively. The differential resistant response of RST-04-105T and RST-04-106T rootstock treatments seen in the Jackson County trials allude to the lack of stable resistance in available germplasm to the prevalent *R. solanacearum* strains from diverse geographic regions (Lin et al. 2008). Additional work is needed to determine if RST-04-106T has broad utility in the Southeast tomato production region.

Hybrid rootstocks, BHN998, BHN1053, and BHN1054, were previously shown to be highly resistant to moderately resistant in artificially inoculated fields in Florida with 19.05%, 28.57%, and 23.81% wilt incidence. In naturally infested field trails in Virginia, similar results were seen with BHN998, BHN1053 and BHN1054 exhibiting lower levels of disease of 10.5%, 5% and 43.5% disease incidence, respectively (McAvoy et al. 2012). In 2012, in Jackson County, NC the BHN998, BHN1053, and BHN1054 rootstocks proved moderately resistance with 45%, 32.5%, and 53.2% disease incidence, respectively. Under lower disease pressure in 2013 the BHN1053 and BHN1054 rootstocks had 37.5% and 27.5% disease incidence, respectively. BHN1054 also conferred superior yields in this study whereas the other two rootstocks did not generate sufficient advantage for consideration in commercial settings.
Three open-pollinated breeding lines, CRA66, HI7996, and HI7998, were adapted as resistant rootstocks in the field production setting around the world (Black et al. 2003; Rivard and Louws 2008; Tikoo et al. 1979). When the breeding lines were tested in open field production in 2012 and 2013 in Jackson County, NC they showed high levels of resistance. CRA66 received 43% and 2.5% disease incidence while HI7998 received 60% and 10% disease incidence in 2012 and 2013, respectively. The breeding line HI7997 was only evaluated in 2013 but had low disease incidence of 7.5%. Previously, CRA66 and HI7996 were reported to confer superior resistance in grafted trials conducted in naturally infested fields in eastern North Carolina compared to severely diseased self and non-grafted controls (Rivard and Louws 2008). The rootstock HI7998 was also evaluated in Florida and exhibited moderate resistance with 53.6% disease incidence in open field conditions (McAvoy et al. 2012). In an inoculation experiment with six *R. solanacearum* strains, one from North Carolina and five from Florida, breeding lines CRA66, HI7996 and HI7998 were found to confer stable resistance (Chellemi et al. 1994). In other regions of the world, it is known there is a differential resistance response of breeding lines CRA66, HI7996, and HI7998 to several *R. solanacearum* strains and resistance is not stable against all strains (Jaunet and Wang 1999; Lin et al. 2008). Also, it has been demonstrated that resistant germplasm may be resistant to a broad diversity of strains or only to specific groups of strains (Matsuzoe et al. 1993). In the case of HI7996, bacterial wilt resistance has been demonstrated as strain specific governed by a major resistance locus (Wang et al. 2000). The similarity of HI7998 resistance response in Florida, Virginia, and North Carolina suggests there may be a relationship between the *R. solanacearum* strains present in Jackson County,
NC and also in Florida and Virginia. *R. solanacearum* has been established for over 100 years in the Coastal plain region of North Carolina (Kelman 1953) while bacterial wilt has only recently emerged as a major issue in western North Carolina tomato production (Rivard et al., 2011). Further studies on *R. solanacearum* strain diversity across North Carolina would be helpful in understanding the differential resistance response of resistant germplasm in the greater Southeast USA. Also, future studies focused on *R. solanacearum* strain diversity in the southeast USA would aid plant breeders in the development of stable resistant germplasm for specific production regions within the southeast. These diversity studies need to be complemented with an understanding of the genes in rootstock selections so that a diversity of genes can be deployed over time (rootstock rotation programs) in order to limit the emergence of strains that overcome resistance in specific rootstocks.

This study provided critical information on rootstocks that have commercial potential to manage bacterial wilt in North Carolina and complements other work accomplished in the Southeast region. Availability of multiple rootstocks to manage bacterial wilt is an important component of IPM programs for the sustainable production of tomato on land infested with *R. solanacearum*. Tomato grafting is becoming a commercial industry in the United States and shows promise for growers to manage this devastating disease and possibly others. Grafting with bacterial wilt resistant rootstocks provides tomato growers an important management tool that will enable the production of susceptible heirloom and large fruiting cultivars on infested soils of North Carolina now and in the future.
LITERATURE CITED


Table 1. Seed Germination Assay.

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<th>5 Days</th>
<th>7 Days</th>
<th>10 Days</th>
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<sup>1</sup> Number of days to grafting is based on germination and growth rate of individual varieties to achieve 2mm stem diameter desired for tube grafting.
Figure 1. Disease progress curves are based on mean disease incidence of four replications per date of observation of each treatment in Jackson County 2012 field trial and included all treatments listed above. Plants were scored as diseased when showing first signs of wilt due to infection by *Ralstonia solanacearum*. 
Figure 2. Mean area under disease progress curve values for Jackson County 2012 included listed treatments in figure legend and were analyzed using the mixed model procedure (PROC MIXED). The Fisher’s protected LSD test (P<0.05) was used for separation of means; treatments with the same letter above each bar are not significantly different from one another.
Figure 3. Total marketable yield was calculated to represent (t/ha). Jackson County 2012 data were analyzed using the mixed model procedure (PROC MIXED). The Fisher’s protected LSD test (P<0.05) was used for separation of means; treatments with the same letter above each bar are not significantly different from one another.
Figure 4. Total yield (marketable weight plus cull weight) were calculated to represent (t/ha). Jackson County 2012 total yield data were analyzed using the mixed model procedure (PROC MIXED). The Fisher’s protected LSD test (P<0.05) was used for separation of means; treatments with the same letter above each bar are not significantly different from one another.
Figure 5. Terminal mean vigor ratings in Jackson County 2012. Data were analyzed using the mixed model procedure (PROC MIXED). The Fisher’s protected LSD test (P<0.05) was used for separation of means; treatments with the same letter above each bar are not significantly different from one another.
Figure 6. Disease progress curves are based on mean disease incidence of four replications per date of observation of each treatment in Jackson County 2013 field trial and included all treatments listed above. Plants were scored as diseased when showing first signs of wilt due to infection by *Ralstonia solanacearum*.
Figure 7. Mean area under disease progress curve values for Jackson County 2013 included listed treatments in figure legend and were analyzed using the mixed model procedure (PROC MIXED). The Fisher’s protected LSD test (P<0.05) was used for separation of means; treatments with the same letter above each bar are not significantly different from one another.
Figure 8. Total marketable yield was calculated to represent (t/ha). Jackson County 2013 data were analyzed using the mixed model procedure (PROC MIXED). The Fisher’s protected LSD test (P<0.05) was used for separation of means; treatments with the same letter above each bar are not significantly different from one another.
Figure 9. Total yield (marketable weight plus cull weight) were calculated to represent (t/ha). Jackson County 2013 total yield data were analyzed using the mixed model procedure (PROC MIXED). The Fisher’s protected LSD test (P<0.05) was used for separation of means; treatments with the same letter above each bar are not significantly different from one another.
Figure 10. Terminal mean vigor ratings in Jackson County 2013. Data were analyzed using the mixed model procedure (PROC MIXED). The Fisher’s protected LSD test (P<0.05) was used for separation of means; treatments with the same letter above each bar are not significantly different from one another.
APPENDIX A- EFFECT OF SOUTHERN STEM BLIGHT DISEASE INCIDENCE ON GRAFTED TOMATOES IN EASTERN NORTH CAROLINA

ABSTRACT

Southern stem blight, caused by *Sclerotium rolfsii*, is a major limiting factor of tomato production in the southeast USA. This soil-borne disease causes a rapid wilting of foliage followed by total plant collapse and complete loss of productivity of a crop under conditions favorable for disease development. Previously, grafting with inter-specific tomato rootstocks demonstrated some level of control in naturally infested soils found in Eastern North Carolina.

Commercially available rootstocks and two breeding lines were evaluated in an onfarm trial conducted in a field with a history of Southern stem blight and Bacterial wilt in Sampson County North Carolina. Southern stem blight first occurred 63 days after transplant (DAT) and persisted for the duration of the trial 101 DAT. Rootstock treatment CRA66, HI7998, and RST-04-105T all reached 100% disease incidence by 101 DAT whereas nongrafted and self-grafted treatments reached 85% and 83% disease incidence, respectively. Rootstock RST-04-106T and three BHN lines (BNH998, BNH1053, and BNH1054) had disease incidence of 83%, 83%, 81%, and 83%, respectively. The highest marketable yields were produced by rootstock treatment CRA66 with the equivalent of 52 t/ha produced. The lowest marketable yield was collected from BHN998 plots with nearly 14 t/ha marketable fruit produced.

Yields of grafted plants were relatively unaffected by Southern stem blight disease and this may be due to the late onset of disease which occurred after fruit set late in the field.
season. Southern stem blight is a major limiting factor in tomato production and these results demonstrate the destructive crop losses that can occur under environmental conditions favorable for disease development. Grafting may be used to combat many soil-borne diseases of tomato and may serve a role in managing *S. rolfsii* under less disease severity than occurred in 2012 in eastern North Carolina.
INTRODUCTION

Southern Stem Blight caused by *Sclerotium rolfsii* is a destructive soil-borne fungal pathogen commonly found in the southeastern USA (Mullin 2001). Southern stem blight is also called Southern blight, and frequently occurs during hot, humid environmental conditions prevalent in many tropical, sub-tropical, and temperate tomato production regions (Mullin 2001). Southern stem blight has a wide host range of 500 plant species including many highvalue horticultural crops like tomato, pepper, and melons (Mullin 2001). The white fan-shaped mycelia are visible on the crown and lower stem of infected tomato plants (Mullin 2001). Symptoms can include water soaked lesions near advancing mycelia, girdled lower stem, chlorosis, blight, and wilt of foliage, quickly followed by total plant collapse (Roberts et al. 2014). *S. rolfsii* produces sclerotia that survive in the soil for extended periods of time in the absence of a host but the pathogen can also overwinter on crop debris and dying plants in the soil (Mullin 2001). The necrotrophic pathogen uses cell wall degrading enzymes and oxalic acid to invade host tissues (Punja 1985). Sclerotia are formed from the mycelial mat and once mature, sclerotia change from a pale brown color to dark brown or tan before they are released to the soil (Roberts et al. 2014). Sclerotia are disseminated via movement of infected transplants, soil, and equipment (Mullin 2001).

Fortunately, there are control tactics available to successfully manage *S. rolfsii* and the primary target for control is sclerotia. Burying sclerotia at the depth of 2 ft. by deep bed plowing can reduce sclerotia survival and deprive the pathogen of light, necessary for sclerotial germination (Punja 1985). Sclerotia germinate at pH of 3 to 7 and are inhibited at pH above 7 (Punja and Gorgan 1982). Raising soil pH to neutral or basic levels can reduce
sclerotia survival because the fungus prefers acidic conditions (Mullin 2001). Crop rotation with non-host crops like corn and small grains for several seasons can reduce inoculum levels in the soil (Roberts et al. 2014). Solarization can help reduce sclerotia inoculum levels but solarization is most effective when combined with chemical or biological control tactics (Elad et al. 1980; Ristaino et al. 1991). Chemical control for *S. rolfsii* relies on soil fumigation with chemicals such as methyl bromide, chloropicrin, PCNB, and methamsodium (Elad et al. 1980; Haas 1976; McCarter et al. 1976; Mullin 2001; Munnecke et al. 1982). Biological microorganisms have demonstrated success in reducing disease incidence including beneficial soil microbes like *Tricoderma* sp., *Bacillus* sp., and *Penicillium* sp. (Punja 1985). *Gliocladium virens*, an antagonistic biological microorganism, was shown to decrease sclerotia numbers as deep as 30cm in the soil when applied after solarization (Ristaino et al. 1991). *Tricoderma koningii* applied to infested soil of potted tomato plants demonstrated highly effective at reducing sclerotia inoculum levels, moreover, the protected plants were more vigorous than non-*Tricoderma*-protected plants (Latunde-Dada 1993). There are few sources of resistance to Southern stem blight in tomato. Southern blight-resistant tomato breeding lines: 5635M, 5707M, 5719M, 5737M, 5876M, and 5913M were developed by Texas A & M University, however, there are no resistant or partially resistant tomato cultivars commercially available (Leeper et al. 1992). Grafting has been shown to reduce disease incidence under high disease severity when inter-specific hybrid rootstocks with *Solanum pimpinellifolium* ancestry were grown in eastern North Carolina (Rivard et al. 2010). The role of grafting to manage *S. rolfsii* in tomato production is unclear and needs further investigation. This study was designed to evaluate rootstock resistance to bacterial
wilt (*Ralstonia solanacearum*) but bacterial wilt did not occur; rather Southern stem blight did. Therefore, the purpose of this study was to assess the potential of grafting to control Southern stem blight in eastern North Carolina.
MATERIALS AND METHODS

A total of seven rootstocks were grafted to one scion cultivar and were evaluated in a grafted trial conducted in Sampson County. Rootstock treatments included two open pollinated breeding lines (CRA66 and HI7998), and several commercial available rootstocks BHN998, BHN1053, BHN1054, RST-04-105T, and RST-04-106T. Non-grafted and selfgrafted scion controls were also evaluated. The susceptible cultivar ‘Fletcher’ was used as a scion in the grafted trial as preferred by the cooperator. Scion and rootstock seeds were sown in Fafard 4P soil media in 72 cell trays. Germination and emergence of the hypocotyl from the media was recorded between 8 to 18 days post-seeding (DPS). Seedlings were grown for 18-28 days to the 2mm stem diameter size, which corresponded to the 3-4 leaf stage and is optimum for grafting (Rivard and Louws 2006). The splice grafting method was used for grafting all rootstock treatments (Rivard and Louws 2006). A healing chamber was constructed for the grafting experiment in the Phytotron at NC State University within a controlled environment growth chamber at 26/22°C +/- 0.5°C (12hr photoperiod, 8ft VHO fluorescent lamps, and standard 100Watt incandescent lighting). The healing chamber design was modeled after previous work (Rivard and Louws 2006) with a large external frame that was wrapped with plastic sheeting, draped with multiple layers of shade cloth, and had a humidifier (Trion 707u Herrmidifier) to direct humidity into the enclose healing chamber to provide moisture for the grafted plants. Grafted plants were healed over 7-10 days with 2 days of complete darkness followed by 3-5 days of gradual increased light while high humidity gradually decreased over the same time interval. Healed plants were gradually
brought back to standard greenhouse conditions and watering conditions. Graft survival was recorded once plants recovered and started growing from the apical meristem.

The on-farm trial was conducted in a field with a history of Southern stem blight and bacterial wilt in Sampson County, NC, part of the coastal plains region of NC. The trial was transplanted May 11th, 2012 and terminated after 101 DAT. The trial was conducted in Ivanhoe, NC (34.613839, -78.247028) and the soil type consisted of Chipley sand (pH=6.0). The site was a certified organic farm that has employed sustainable natural management of the land for over 20 years. Plants were spaced at 53cm in-row with 1.5m between-row spacing (Rivard et al. 2010). Plants were transplanted at the soil line of the seedling cells, which corresponded to the grafting union remaining 2.5-3cm above the soil line. Plants were managed using a standard stake and weave system. Plants were pruned below the graft union to prevent rootstock sucker growth and were pruned up to the third axil from the graft union. The trial was managed according to grower experience and certified organic practices. Observations were recorded on a weekly to biweekly basis and included disease incidence, plant height, plot vigor, and eventually yield as fruit matured. Southern stem blight disease incidence was scored based on the number of plants with visible white mycelial mats present at the crown or lower stem of infected plants that also exhibited wilt symptoms. Destructive plant samples were collected after plants were scored as dead to verify the pathogen and cause of death. Plant height (cm) was collected on a weekly basis after 14 DPT by measuring from the base of the plant at the soil line up to the growing point of the main stem. Plot vigor ratings were assigned to each plot based on a subjective rating scale from 1 to 10, with 1 representing a poor looking plot with mostly dead plants and a rating of 10 indicating
healthy, good looking plots. Plots were harvested as fruit matured and yield was recorded 5 times toward the end of the field season.

A randomized complete block design was used for the field trial. Twelve plant plots were desired for the 2012 trial but lower numbers of plants were planted for specific treatments (4-12 plants/plot) due to inconsistent graft survival rates. Treatments were replicated 4 times in each experiment. All disease incidence data were normalized according to plant stand in each plot. Data were analyzed using the mixed model procedure (PROC MIXED) in SAS v9.4 (SAS Institute, Cary, NC) with Enterprise Guide 6.1 (SAS Institute, Cary, NC). Mean differences in rootstock effects were separated with Fisher’s protected LSD test using a significance level of P=0.05. Disease progress curves were created from disease incidence data and area under the disease progress curve (AUDPC) values were calculated and analyzed with the mixed model procedure. Total yield (marketable and cull weights) and marketable yield were analyzed using the mixed model procedure and calculated to represent tons/hectare (t/ha) in the figures.
RESULTS

There was substantial and uniform disease pressure due to *S. rolfsii* in 2012. Southern stem blight was first observed 63 DAT and disease incidence quickly became widespread in the field. The fungal pathogen was confirmed by incubation of infected plant tissue in humidity boxes and observations of characteristic mycelial growth and formation of sclerotia.

There were significant differences in total disease incidence between rootstock treatments, which ranged from 81-100% (P<0.0001). The highest disease incidence was seen in treatments CRA66, HI7998, and RST-04-105T with 100% disease incidence by 101 DAT (Figure 1). By 74 DAT, rootstocks CRA66, HI7998, and RST-04-105T already displayed the highest disease with 100%, 79%, and 63% incidence, respectively (P=0.0284). The least disease incidence was found in the self-grafted treatment, RST-04-106T, BHN1054, BHN998, BHN1053, and non-grafted treatments with 83%, 83%, 83%, 81%, 83%, and 85% disease by 101 DAT, respectively. The AUDPC values for Southern stem blight also showed significant differences between rootstock treatments (P<0.0001, Figure 2). Differences in means were separated with Fisher’s protected LSD test (P=0.05) and showed rootstocks RST-04-106T, BHN998, BHN1053, BHN1054, and non-grafted and self-grafted treatments had reduced disease compared to rootstocks CRA66, HI7998, and RST-04-105T (Figure 2).

Total yields and marketable yields of grafted treatments were impacted by treatments (Figure 3). The highest marketable yields were produced by rootstock treatment CRA66 with the equivalent of 52 t/ha. The lowest marketable yield was collected from BHN998 plots with 13.67 t/ha produced (Figure 3). The percent of marketable fruit yield produced by grafted
treatments were also impacted by treatment (P=0.0360, Figure 4). The treatment with the highest percent marketable yield was CRA66 with 71.4% marketable fruit produced. The treatment that produced the lowest percent marketable fruit was BHN998 with 37.5% marketable fruit produced.

Plant height was not an informative parameter for evaluating the grafted treatments and there were no significant differences between rootstocks. The highest numeric terminal plant height was seen in rootstock BHN998 with a mean height of 73cm. The shortest plants were measured in BHN1053 grafted plots with a mean terminal plant height of 58.3cm. Plot vigor was subjectively assigned to plots based on overall plot appearance according to a 1-10 scale and differences in plot vigor rates of the grafted treatments was observed (P>0.0001; Figure 5). The most vigorous rootstocks were BHN998, BHN1053, BHN1054, RST-04-105T and RST-04-106T during the experiment and received the highest mean terminal ratings of 6.8, 6.8, 6.8, 6.8, and 6.5, respectively. The least vigorous treatments were reliably the nongrafted and self-grafted controls with mean terminal vigor ratings of 4 and 3, respectively.
DISCUSSION

There was high Southern stem blight disease present in the 2012 field trial in Sampson County NC. This trial provided insight to the performance of grafted treatments for field production in naturally infested soil conditions and demonstrated that yields were relatively unaffected by disease due to the late onset of disease. Disease developed during mid-summer and was initially observed during the first harvest, 63DAT, and quickly became widespread by 76DAT with 81-100% disease incidence in all grafted treatments. The rootstock that produced the most yield was CRA66, which was also the most susceptible to Southern stem blight with 100% disease incidence. The rootstocks with the least disease incidence included RST-04-106T, BHN1053, BHN998, and BHN1054 which may have some resistance to Southern stem blight. Grafting with inter-specific hybrid rootstocks such as Beaufort, Big Power, and Maxifort showed reduced Southern stem blight disease incidence in North Carolina fields with high disease pressure between 2006 and 2008 (Rivard et al. 2010). Southern stem blight resistance in tomato was first found in *Solanum pimpinellifolium* and 23 years later six resistant lines were released from Texas A&M University, but since then there have not been new Southern stem blight resistant cultivars available (Leeper et al. 1992; Mohr and Watkins 1959). Tomato disease resistance to *S. rolfsii* needs further evaluation to identify good sources of resistance and inter-specific hybrids such as the Texas A&M lines and hybrid rootstocks may prove to be the best resources to examine first.
LITERATURE CITED


Figure 1. Southern stem blight disease progress curves are based on mean disease incidence of four replications per date of observation of each treatment in Sampson County 2012 field trial and included all treatments listed above. Plants were scored as diseased when showing first signs of wilt due to infection by *Sclerotium rolfsii*. 
Figure 2. Mean area under disease progress curve values for Sampson County 2012 included listed treatments in figure legend and were analyzed using the mixed model procedure (PROC MIXED). The Fisher’s protected LSD test (P<0.05) was used for separation of means; treatments with the same letter above each bar are not significantly different from one another.
Figure 3. Total marketable yield was calculated to represent (t/ha). Sampson County 2012 data were analyzed using the mixed model procedure (PROC MIXED). The Fisher’s protected LSD test (P<0.05) was used for separation of means; treatments with the same letter above each bar are not significantly different from one another.
Figure 4. Percent (%) marketable yield was analyzed using the mixed model procedure (PROC MIXED). The Fisher’s protected LSD test (P<0.05) was used for separation of means; treatments with the same letter above each bar are not significantly different from one another.
Figure 5. Terminal plot vigor ratings in Sampson County 2012. Data was analyzed using the mixed model procedure (PROC MIXED). The Fisher’s protected LSD test (P<0.05) was used for separation of means; treatments with the same letter above each bar are not significantly different from one another.
APPENDIX B - EFFECT OF BACTERIAL SOFT ROT, CAUSED BY

PECTOBACTERIUM CAROTOVORUM SUBSP. CAROTOVORUM, ON GRAFTED TOMATOES IN NORTH CAROLINA

ABSTRACT

Bacterial soft rot caused by *Pectobacterium carotovorum* subsp. *carotovorum* is a major post-harvest disease of many fruit and vegetable crops. This bacterial pathogen can also cause stem rot diseases and contribute to large crop losses in cabbage, potato, and tomato. The opportunistic pathogen occurs worldwide and is most frequently found in tropical, sub-tropical and warm temperate regions. Although bacterial soft rot of tomato is not a major limiting biotic factor in North Carolina, it can impact crop productivity and limit yields produced by infected plants. Bacterial soft rot was present in 2013 in an on-farm grafted tomato trial conducted in the piedmont of North Carolina. Bacterial soft rot of tomato stem tissue was observed 28 days after transplant (DAT) and persisted for the duration of the experiment. Disease incidence ranged from 5% to 55% with the self-grafted ‘Florida 47’ control showing the least disease incidence and Maxifort rootstock treatment having the most disease, respectively. Yields were impacted by the presence of bacterial soft rot disease and ranged from 38.9 t/ha to 21.66 t/ha in the moderately infected BHN1053 rootstock plots and severely infected Maxifort grafted plots, respectively. The results from this field trial are not conclusive and suggest a need for further evaluation of tomato rootstocks with resistance to bacterial soft rot.
INTRODUCTION

*Pectobacterium carotovorum* subsp. *carotovorum* causes bacterial soft rot of many vegetable and horticultural crops such as tomato, potato, carrots, irises, cabbage, and leafy greens (Davis 2002). Bacterial soft rot is predominately a post-harvest disease of fruit and vegetable crops (Davis 2002). It is not a major disease of tomato but when the disease is present it causes large amounts of crop damage. Symptoms of bacterial soft rot include water soaked lesions, wilting and complete plant collapse (Daughtrey et al. 2006). Stem lesions may be dark and discolored and a transverse section of the stem lesion may reveal a discolored pith. The water soaked stem lesions will become slimy and may secret bacterial ooze at the tissue surface (Davis 2002). The weak bacterial pathogen requires a predisposed or susceptible plant for invasion via wounds and natural openings under high humidity conditions (Daughtrey et al. 2006). Once the bacterium has become established on plants it can move systemically or be splash dispersed to leaves and eventually to fruit to cause postharvest soft rot (Kucharek and Bartz 1981). The disease most frequently occurs during periods of extended rainfall. Favorable temperatures for disease development range from 20-25°C (Davis 2002). The bacterium can be spread via contaminated equipment, infected transplants, or infested soils. However, heavy rainfall is a major mechanism of dissemination from plant to plant under field conditions (Daughtrey et al. 2006).

Bacterial soft rot of stem tissue is most common on stalked vegetables and can be controlled by many cultural management tactics (Kucharek and Bartz 1981). One method is to avoid planting in areas with the potential for high soil saturation and select field sites with sufficient drainage. Proper plant maintenance should be conducted when the plants are dry,
and pruning should be conducted often to avoid removal of large suckers and large wound sites for the pathogen to enter (Kucharek and Bartz 1981). Crop rotation with non-hosts such as corn, snap beans and beets can reduce disease pressure (Lunt 2013). Sanitize field equipment often with 70% alcohol or a 10% bleach solution and avoid touching infected plant material before working in uncontaminated field sites (Lunt 2013). Fertilize with low nitrogen rates as high fertility soils promote *Pectobacterium*. Resistance breeding efforts are primarily focused on post-harvest disease control. There are a few resistant varieties including Florida MH-1, FlorAmerica, Flora-Dade, and Homestead 24 which all produce soft rot resistant fruit (Kucharek and Bartz 1981). Fruit stem scars are common infection courts for bacterial soft rot in post-harvest fruit because water uptake occurs at the stem scar site for a 4 hr period of time post-harvest. Water baths are used for processing fruit where the fruit skin is infiltrated with water. Post-harvest bacterial soft rot disease incidence can be reduced by postponing water infiltration for 4 hr post-harvest when stem scars become congested with air (Smith et al. 2006) or floating fruit in baths where the water is 5-6 °C above the ambient fruit temperature. Low stem scar water uptake is associated with tolerance to bacterial soft rot and some tolerant varieties include Solar Set, Equinox, Escudero, Solar Fire, Sebring, Sanibel, and NC84173 (Smith et al. 2006). Grafting is not a management tactic currently used to control bacterial soft rot of tomato as this disease is mostly a post-harvest concern for growers; however, the process of grafting may introduce the bacteria and highly vegetative plants may be more susceptible. The purpose of this experiment was to document the incidence of bacterial soft rot as impacted by rootstock treatment. The
experiment was designed to assess bacterial with resistance but bacterial wilt did not occur and instead a high incidence of bacterial soft rot was observed.
MATERIAL AND METHODS

A total of eight rootstocks were grafted to one scion cultivar for the field evaluations and included HI7997, HI7998, BHN1053, BHN1054, Maxifort, RST-04-105T, RST-04106T, and Cheong gang. The susceptible cultivar Florida 47 was used as a scion in the grafted trial as preferred by the cooperator. Non-grafted and self-grafted Florida 47 control treatments were also evaluated. Seedlings were grown for 18-28 days to the 2mm stem diameter size which corresponded to the 3 to 4 leaf stage and is optimum for grafting (Rivard and Louws 2006). The splice grafting method was used for grafting rootstock treatments (Rivard and Louws 2006).

A healing chamber was constructed in a shaded, cool greenhouse unit with an existing overhead mist system in the Mary Ann Fox Greenhouse. The healing chamber was assembled by draping the external mist bench frame with multiple layers of shade cloth in a 3.05mx1.2m mist bench area. The mist bench was automated to apply mist at 8 min intervals with 4 s mist durations for a 12 hour period during daylight hours (CoolNet Pro, cross four nozzle configuration, 65 micron average droplet size, output ~7.5-14mL/nozzle unit/mist event, Netafim). The overhead mist system supplied fine mist through nozzles suspended 45cm above grafted plants and spaced 45cm apart, attached to PVC piping from the topmost support of the external bench frame. Mist frequency was decreased from 8 min intervals to 12 min intervals with 4 s mist during the healing process to reduce water droplet formation and droplet accumulation on leaf surfaces. Additionally, mist frequency was increased to 4min intervals with 4 s mist to reduce drought stress when observed.
Grafted plants were healed over a 7 to 10 day period by gradually removing layers of shade cloth from the chamber day by day then adjusting the misting conditions over several days to acclimate plants to standard greenhouse conditions. Grafted plants were hardened off in greenhouse conditions for 7 days prior to transplant. Grafting survival was recorded prior to transplant.

The on-farm trial was conducted in a field with a history of Bacterial wilt in the piedmont region of NC. The trial was transplanted May 23rd, 2013 and extended for 85 DAT. The experiment was conducted on a commercial farm, in Willow Spring, NC (35.593305, -78.711020) with Norfolk loamy sand (pH=6.5) (Soil Survey, USDA). Plants were spaced 46cm apart in row and 1.5m between rows. Plants were transplanted at the soil line of the seedling cells which corresponded to the graft union remaining 2.5-3cm above the soil line. Plants were managed using a standard stake and weave system. Plants were pruned below the graft union to prevent rootstock sucker growth and were pruned up to the third axil from the graft union. Fertilizer and pest management practices were performed using standard recommendations (Ivors et al. 2010).

Observations were recorded on a weekly to biweekly basis and included disease incidence, plant height, plot vigor, and harvest at the conclusion of the study. Bacterial soft rot disease incidence was scored based on the number of plants in each plot that exhibited wilt symptoms caused by *Pectobacterium carotovorum* subsp. *carotovorum*. Destructive plant samples were collected after plants were scored as dead to verify the pathogen and cause of death. Plant height (cm) was collected on a weekly basis starting 14 DPT by measuring from the base of the plant at the soil line up to the growing point of the main
stem. Plot vigor ratings were assigned to each plot based on a subjective rating scale from 1 to 10, with 1 representing a poor looking plot with mostly dead plants and a rating of 10 indicating healthy, good looking plots. Plots were harvested as fruit matured and yield was recorded 5 times.

A randomized complete block design was used for the field trial. Ten plant plots were planted for each treatment. Treatments were replicated 4 times in the experiment. All data were analyzed with SAS v9.4 (SAS Institute, Cary, NC) with Enterprise Guide 6.1 (SAS Institute, Cary, NC). Data was analyzed using the mixed model procedure (PROC MIXED) and mean differences in rootstock effects were separated with Fisher’s protected LSD test using a significance level of P=0.05. Disease progress curves were created from disease incidence data and area under the disease progress curve (AUDPC) values were calculated and analyzed using the mixed model procedure. Total yield (marketable and cull weights) and marketable yield were analyzed using the mixed model procedure and calculated to represent tons/hectare (t/ha) in the figures.
RESULTS

The field site was selected based on grower experience with extensive bacterial wilt pressure. However, no bacterial wilt disease occurred in this trial, though disease incidence was observed in adjacent field locations. However, bacterial soft rot was present and the subsequent bacterial soft rot disease incidence was observed from 28DPT to the end of the experiment. Plant samples were submitted to the Plant Disease and Insect Clinic at NCSU and the causal agent was verified as *Pectobacterium carotovorum subsp. carotovorum*. Disease incidence was not different among rootstock treatments at the first observation of disease at 28DAT with the first incidence observed in BHN1053 (Figure 1). By 40 DAT, higher disease incidence was observed in Maxifort than the other rootstock treatments (P=0.048; Figure 1). Disease incidence was significantly less with the self-grafted control compared to Maxifort and Non-grafted control treatments at 49 DAT (P=0.0046, Figure 1). The self-grafted control, HI7998, and Cheong gang treatments had the lowest level of disease with a final incidence level of 5%, 10%, and 10%, respectively, whereas the Maxifort rootstock treatment and the non-grafted control had significantly more disease with a final incidence of 55% and 28%, respectively (P=0.0111; Figure 1). The AUDPC values showed differences among rootstock treatments. Disease incidence was highest with Maxifort, among all of the rootstock treatments (P=0.0024; Figure 2). The Self-grafted control had the least amount of disease over the course of the experiment.

Total and marketable yields were impacted by rootstock treatments (Figure 3; 4). The highest numeric marketable yield was collected from Self-grafted control plots with 16 t/ha fruit produced. The Maxifort rootstock plots produced the least marketable fruit yields of
8.78 t/ha (Figure 4).

There were significant differences in plant height among rootstocks early in the field season. At 14 DAT, BHN1053 was significantly taller in comparison to BHN1054, Cheong gang, Self-grafted, Non-grafted and Maxifort treatments with 41.0cm, 33.0cm, 28.4cm, 24.75cm, 23.3cm, and 14.5cm, respectively (P<0.0001). By 35DAT, RST-04-105T was the tallest treatment with 95.0cm and was significantly greater than Maxifort plots with 59.3cm growth, respectively (P=0.0447). The terminal plant heights, collected at the last observation on 56DAT, were not significantly different among rootstock treatments. Plot vigor ratings were significantly different before disease occurred on 28DAT. However, there were no significant differences in vigor rating between rootstock treatments at 35DAT to the end of the experiment, 85DAT. The non-grafted treatment was most vigorous and significantly more vigorous than rootstock treatments BHN1054 and Maxifort (P<0.0001) at 14DAT. The non-grafted plots were still significantly more vigorous at 28 DAT than all other rootstock treatments with the least vigorous treatments including rootstocks BHN1053 and RST-04106T (P=0.0073). Terminal vigor ratings were not statistically significantly different among rootstock treatments at 62 DAT.
DISCUSSION

The trial was conducted in a field with a history of bacterial wilt disease from continuous tomato crops year after year. However, there was no bacterial wilt disease pressure in 2013 in the field site selected for the grafted trial. The presence of *Pectobacterium carotovorum subsp. carotovorum* and the susceptibility of the rootstock/scion treatments to bacterial soft rot could be attributed to several factors including weather, soil fertility, heavy pruning, improper sanitation of pruning equipment, and host susceptibility. There was an excessive amount of rain fall in 2013 compared to 2012, evident with June mean rainfall in 2012 of 1.055” compared to the 5.145” experienced in June 2013 (Cedar Lake Venture, Inc. 2013; Cedar Lake Venture, Inc. 2014). The wet weather and nutrient rich soils from drip applied fertilizer could have contributed to the bacterial soft rot disease incidence as these are favorable conditions for disease development (Stall 1991). The plants were heavily pruned at 22 DAT from the third sucker to the soil line due to excessive plant growth and this created wound sites for the opportunistic pathogen to enter susceptible plant hosts. The transmission of bacteria through mechanical damage caused during pruning could have been a major contributing factor in bacterial soft rot disease incidence (Stall 1991). The pruning shears were sanitized with 70% ethanol between plots. Nevertheless, this may not have been sufficient in preventing the spread of the bacteria from plant to plant. The rootstocks treatments used for the trial impacted the levels of bacterial and Maxifort had the highest disease incidence of all grafted treatments. The Maxifort grafted plants tended to be more vegetatively vigorous than other rootstock treatments and required more lateral branch pruning, thus created more wound sites for the pathogen to invade during pruning and
high vegetative growth may have enhanced susceptibility. Although bacterial soft rot is typically not a major disease in the field, its re-emerging occurrence in high fertility soils suggests a need for further investigation. The results from this field trial are not conclusive but allude to future research opportunities in the area of bacterial soft rot and impact of rootstock selection.
LITERATURE CITED


Figure 1. Soft rot disease progress curves are based on mean disease incidence of four replications per date of observation in Wake County in 2013 and included all treatments listed above. Plants were scored as diseased when showing first signs of stem rot caused by *Pectobacterium carotovorum ssp. carotovorum*.
Figure 2. Mean area under disease progress curve values for Wake County 2013 included listed treatments above and data were analyzed using the mixed model procedure (PROC MIXED). The Fisher’s protected LSD test (P<0.05) was used for separation of means; treatments with the same letter above each bar are not significantly different from one another.
Figure 3. Total yield (t/ha) was analyzed with the mixed model procedure (PROC MIXED). The Fisher’s protected LSD test (P<0.05) was used for separation of means; treatments with the same letter above each bar are not significantly different from one another.
Figure 4. Total marketable yield (t/ha) was analyzed using the mixed model procedure (PROC MIXED). The Protected Fisher’s LSD test (P<0.05) was used for separation of means; treatments with the same letter above each bar are not significantly different from one another.