The Spatial and Temporal Distribution and Management of Tomato Bacterial Wilt on Virginia's Eastern Shore

Adam Francis Wimer

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science
In
Plant Pathology, Physiology, and Weed Science

Steven L. Rideout
Henry P. Wilson
Joshua H. Freeman
Charles S. Johnson
Jonathan D. Eisenback
Boris A. Vinatzer

December 9, 2009
Painter, VA

Keywords: *Ralstonia solanacearum*, ordinary runs, resistant cultivar, acibenzolar-*S*-methyl, grafting

The Spatial and Temporal Distribution and Management of Tomato Bacterial Wilt on Virginia's Eastern Shore

Adam Francis Wimer

ABSTRACT

In 2007 and 2008 more than 100 million dollars of fresh market tomatoes were grown in Virginia, with the majority of production occurring on the Eastern Shore of Virginia (ESV), according to the National Agricultural Statistics Service. Bacterial wilt of tomato, caused by *Ralstonia solanacearum* (Smith) and Yabucchi *et al.*, is the most devastating disease of tomato on the ESV. Four 'observational trials' were conducted on the ESV over three growing seasons to determine the temporal and spatial distribution of this disease in commercial tomato fields. Plants were assessed at approximately one-week intervals throughout the growing seasons and the incidence of bacterial wilt for each individual plant was recorded. A steady increase in both disease incidence and clustered distribution of the disease within rows was observed as the growing season progressed. Positive correlations between disease incidence and percentage of rows exhibiting a significant clustered distribution occurred in all trials, which indicated an increase in clustered distribution as disease incidence increased.

Research trials were conducted over three years, beginning in the summer of 2007, to investigate the effects of tomato bacterial wilt resistant cultivars on the ESV. In 2008 and 2009, the selective, systemic compound which induces host plant resistance, acibenzolar-*S*-methyl (ASM) was incorporated into resistant cultivar trials. Results from the 2007 trial revealed significant resistance in some of the breeding lines, CRA 66 and PI 126408. The 2008 and 2009 trials revealed that ASM was not effective at reducing levels of bacterial wilt. Grafted transplants in the spring trials of 2008 and 2009 had varied results in resistance and yield.

Results revealed the tomato cultivar BHN 669 was an excellent resistant cultivar with promising yield potential and fruit quality.

Acknowledgements

I would like to extend a grateful thank you to my major advisor Dr. Steve Rideout, for his guidance and patience throughout my graduate studies and while I conducted my research. I would also like to thank the rest of my committee, Drs. Charles Johnson, Jonathan Eisenback, Boris Vinatzer, Joshua Freeman, and Henry Wilson. With the help and guidance from my major advisor and the rest of my committee members I was able to gain a better understanding of the research process and its demands.

I would also like to thank Dr. Jay W. Scott from the University of Florida for providing the seed for the resistant cultivar trials I conducted. Also I am appreciative to the commercial tomato producers on Virginia's Eastern Shore, East Coast Brokers and Packers and Six L's Packing Company, for allowing me to conduct portions of my research within their fields. I would also like to thank the USDA-CSRESS Methyl bromide alternatives (2008-51102-04486), Virginia Agricultural Council and the United Phosphorus Inc. for providing the funding that allowed me to conduct my research.

I am also very thankful for Christine Waldenmaier, Plant Pathology technician. Her insight and guidance helped me tremendously while I conducted my trials. I would like to extend my thanks to the Plant Pathology summer crew for helping me conduct my trials throughout summer and fall field seasons. I would also like to extend a thank you to the rest of the faculty and staff at Virginia Tech's Eastern Shore Agricultural Research and Extension Center.

Last and most importantly, I would like to thank my parents Tim and Gayle Wimer for supporting me and believing in me no matter what throughout my life. Without you this would not have been possible for me. You have always picked me up when I needed a little help even if I did not know it. I am forever grateful for your love and encouragement.

TABLE OF CONTENTS:

List of Tables:

Chapter 1: Introduction

Chapter 2: Temporal and spatial distribution of tomato bacterial wilt on Virginia's Eastern Shore

Chapter 3: Management of tomato bacterial wilt with grafted transplants, resistant cultivars and breeding lines in conjunction with acibenzolar-S-methyl

List of Figures:

Chapter 1: Introduction

Chapter 2: Temporal and spatial distribution of tomato bacterial wilt on Virginia's Eastern Shore

Chapter 3: Management of tomato bacterial wilt with grafted transplants, resistant cultivars and breeding lines in conjunction with acibenzolar-S-methyl

Chapter 1:

Introduction:

Although a wide variety of vegetable crops are produced on the Eastern Shore of Virginia (ESV), fresh market tomatoes (*Solanum lycopersicum* L.) are the most valuable crop. In 2007 more than 55 million dollars of fresh market tomatoes were produced making Virginia the third ranked state in the country in fresh market tomato production (64). Approximately 90% of Virginia's tomato production comes from two counties on the ESV, Accomack and Northampton (64).

The scarcity of land is a major limitation of tomato production on the ESV because it limits crop rotations which make soilborne diseases more likely and problematic. Tomato fields are in production continuously because of the escalating costs of land and the additional need for irrigation ponds and wells for drip irrigation. This monoculture production system is perfect for persistent soilborne pathogens like *Ralstonia solanacearum* (Smith) Yabuuchi *et al.*, the causal agent of tomato bacterial wilt. In many ESV tomato fields, *R. solanacearum* is present and causes severe yield losses every year. Suppressing levels of this pathogen is imperative to sustain fresh market tomato production on the ESV.

Organism:

Bacterial wilt is a devastating disease of many solanaceous crops grown in temperate regions. Bacterial wilt is also known as southern bacterial wilt, solanaceous wilt and southern blight (58). *Ralstonia solanacearum* is a gram negative bacterium with 1-4 polar flagella; it is aerobic, catalase and oxidase positive, and forms nitrites from nitrates (58). *Ralstonia solanacearum* is an extremely resilient pathogen, persisting in irrigation ponds and other surface water where susceptible aquatic weed hosts are found. Elphinstone *et al.* (19) indicated two

outbreaks of potato bacterial wilt were associated with contaminated irrigation water. The pathogen was recovered in higher concentrations in irrigation water when samples were taken downstream of the susceptible weed host bittersweet (*Solanum dulcamara* L.) (19). Similarly, researchers in Florida indicated *R. solanacearum* was present in northern Florida tomato irrigation ponds during the months of May to October (42). In this study the pathogen was associated with dominant weed hosts that grew around these irrigation ponds such as Pennsylvania smartweed (*Polygonum pennsylvanicum* L.) and water pennywort (*Hydrocotyle ranunculoides* L.f.) (42).

Ralstonia solanacearum forms two types of colonies when grown on a semi-selective tetrazolium medium: one is small, flat, red, and butyrous; and the other is large, elevated, mostly white with light pink centers, and fluidal (58). The organism grows most favorably in temperatures of 35 - 37^{o}C, but can survive in tomato plants across a temperature range of 10 - 41^{o}C (38, 58). In a study by Gallegly and Walker (24), tomato plants were inoculated with *R. solanacearum* and then subjected to different soil and air temperatures for 30 days after inoculation and the incidence of wilt was recorded. This research showed an increase in bacterial wilt in tomato plants subjected to warmer soil temperatures from 22 ^{o}C to 36 ^{o}C and warmer air temperatures from 16 ^{o}C to 28 ^{o}C (24).

Ralstonia solanacearum is classified into five biovars and five races based on *R. solanacearum*'s metabolism of select disaccharides and hexose alcohols and organism host range (4, 8, 9, 38, 39, 40, 58). The races are organized by host range (Table 1): race 1 affects tobacco (*Nicotiana tobacum* L.), tomato (*Solanum lycopersicum* L.), and many solanaceous and other weeds. Race 2, known as moko disease, occurs on triploid bananas (*Musa* spp. L.). Race 3 causes bacterial wilt on tomato and potato (*Solanum tuberosum* L.), but is not very virulent on

other solanaceous crops (10). Race 4 causes bacterial wilt of edible ginger (*Zingiber officinale* Roscoe) and race 5 causes bacterial wilt on mulberry (*Morus* spp. L.) (21, 39). Ornamental hosts such as the Bird of Paradise (*Strelitzia reginae* Aiton) are mainly affected by race 1 (36). Race 1 is endemic to the southeastern United States and is the race present on the ESV.

The biovar classification system (biovars 1-5) is based on the organism's ability to utilize three disaccharides (maltose, lactose, and cellobiose) and oxidize three hexose alcohols (mannitol, sorbitol, and dulcitol) (37, 91) (Table 2). The biovar classification system is only intended for epidemiological classifications rather than taxonomical purposes (37). The biovar separation based on metabolic properties separates the pathogen into two groups, biovars 1 and 2 as one group, and biovars 3, 4, and 5 as a second group (37). Biovars 3, 4, and 5 are more nutritionally diverse than biovars 1 and 2 (37). Recent research has classified *R. solanacearum* into the same two major clusters based on restriction fragment length polymorphism and 16S rDNA sequences (21).

More recently *R. solanacearum* has been classified as a species complex and characterized into four major phylotypes based upon geographical origin and according to DNA sequence analysis (Table 3) (20). Phylotype I contains biovars 3, 4, and 5 which are primarily isolated from Asia (20). Phylotype II contains biovars 1, 2, and 2T (a tropical variant of biovar 2) isolated primarily from the United States. Phylotype III contains biovars 1 and 2T, but unlike phylotype II, these strains are primarily isolated from Africa and the surrounding islands (20). Phylotype IV contains biovars 1, 2, and 2T, but these strains are isolated primarily from Indonesia, and are also found in Australia and Japan (20).

Symptoms:

The initial symptom of bacterial wilt on tomato is the loss of turgidity in one or two upper leaves (58). Under favorable environmental conditions, the entire infected plant rapidly wilts within two to three days of the initial symptoms (Fig. 1) (58). In the early stages of disease, the vascular tissue becomes yellow, eventually turning dark brown. In advanced stages of the disease, the pith and cortex also become brown (58). Other symptoms include bacterial streaming, which can be observed when stems are cross sectioned and partially submerged in water. Bacterial exudate streams from the cut ends of the stems submerged in water appearing as a milky fluid.

Figure 1. Field picture of tomato bacterial wilt.

Host Range:

Ralstonia solanacearum can infect more than 200 plant species representing 44 families (38, 39, 58, 59, 73). The most economically important crops infected are tomato, potato, tobacco, banana and eggplant (*Solanum melongena* L.) (58). *Ralstonia solanacearum* can also survive on cashew (*Anacardium occidentale* L.), custard apple (*Annona* spp. L.), Alexandra palm (*Archontophoenix alexandre* (F. Muell.) H. Wendl. & Drude), sweet potato (*Ipomoea batatas* Lam.), cassava (*Minhot esculenta* Crantz), Eucalyptus (*Eucalyptus globulus* Labill.), peanut (*Arachis hypogaea* L.) in the United States and others (1, 17, 38, 57, 59, 61, 87). Certain weed

hosts of *R. solanacearum*, such as horsenettle (*Solanum carolinense* L.), show no symptoms of infection making detection in fallow fields problematic (18, 33, 38, 94).

Disease Cycle:

Interactions between tomato plants and *R. solanacearum* can be influenced by numerous factors. The organism can enter the plant through root wounding from cultivation, insect damage, nematode feeding sites, or through natural openings from emerging secondary roots (38, 45, 48, 56, 58, 80, 88). In a study by Napiere and Quimio (63), co-inoculation with root-knot nematodes *Meloidogyne incognita* (Kofoid & White, 1912) Chitwood, 1949 and *R. solanacearum* caused bacterial wilt to occur in earlier growth stages, was more severe, and significantly decreased yield in susceptible and resistant plants. Once present within the roots, *R. solanacearum* travels into the vascular tissue through the xylem, where it rapidly multiplies (58). The organism begins to clog the vascular tissue with the production of extracellular polysaccharides, restricting the movement of nutrients and water throughout the plant, eventually causing the characteristic wilting symptoms (16). Research examining virulence factors among R. solanacearum strains revealed copious amounts of extracellular polysaccharides present among virulent strains that caused wilt and absent among avirulent strains that did not cause wilt (16).

Ralstonia solanacearum can spread throughout infested fields and be introduced into new fields by several vectors. Within an infested field, the bacteria can spread through soil and water movements. This pathogen has polar flagellum that allow it to be motile in water. Researchers Mao and He (54) found that motile strains of this pathogen migrate towards susceptible roots. They observed that motile strains cause disease more quickly, and with more severity than non-motile, virulent strains (54). *Ralstonia solanacearum* has the ability to migrate towards

susceptible root tissues, making it especially proficient at causing disease in soils with high moisture content. Mao and He observed that the pathogen is immotile when exiting plant roots; however, once exposed to water, the pathogen regained full motility after 5 hours (54).

Rainfall, irrigation, and field preparation or maintenance equipment can readily spread the bacterium. *Ralstonia solanacearum* can be introduced to new fields through infected transplants, movement of soil runoff, or farm machinery, making long distance distribution possible. *Ralstonia solanacearum* is able to survive in potato tubers and to be carried on vegetative propagating material (38). Potato tubers latently infected with *R. solanacearum* have been shipped globally, facilitating the spread of the disease across nations (8, 12, 13, 38, 67, 90).

Soil temperature and moisture can have a profound effect on incidence and severity of bacterial wilt. Gionson-Monsalud *et al*. (31) observed that soil moisture and temperature are the most important factors for the survival of *R. solanacearum*. Severity of bacterial wilt is greatest in soils that are well drained, but have good water retention properties. Bacterial wilt resistance found in tomato cultivars is temperature and strain specific (38, 51, 53, 60, 79, 92). Researchers have indicated that resistance can begin to break down in plants as the temperature increases, and the pathogenicity of some strains of the pathogen can increase as the temperature increases (38, 51). A break down in resistance while there is an increase in pathogenicity could cause marked differences in resistant cultivars at varying temperatures and locations. Krauz and Thurston (51), showed that an environment with a temperature of 32^{o}C significantly increased bacterial wilt compared to the control temperature of 26.6^{o}C on two resistant tomato cultivars. The results from this research indicated an increase in the pathogenicity of *R. solanacearum* as the temperature increased (51). Robertson (83) found that a mean minimum temperature of 15^{o}C had to be exceeded for disease to occur within a growing season.

The most common environmental factor that promotes bacterial wilt worldwide is high soil moisture levels (53). Research by Robertson (83) showed the onset of disease to occur 18 days earlier in two trials during seasons with increased rainfall thus leading to increased soil moisture. Buddenhagen and Kelman (9) cited four ways in which high soil moisture affects bacterial disease levels, through: 1.) increased survival of the bacterium in the soil, 2.) increased initial infections, 3.) increased disease development within plants after infection, and 4.) increased spread throughout the soil.

The development and survival of *R. solanacearum* is favored by higher soil moisture in well drained soils, but in soils more prone to flooding and soil desiccation or drying, the survival of *R. solanacearum* is hindered (9, 38, 48). Soils that encounter flood and drought cycles, as well as river flood plains, which experience extreme heat when dry, are generally free of bacterial wilt (38, 84). Soils with high calcium levels have reduced levels of bacterial wilt (38, 75).

Management:

Bacterial wilt is difficult to control in tomato. To successfully manage a soilborne pathogen such as tomato bacterial wilt, an integrated pest management (IPM) strategy is often the most efficient production plan. Much of the research on control of bacterial wilt focuses on using resistant cultivars and fumigation. With the imminent phase out of methyl bromide (MBr), fumigant alternatives are being examined for weed, nematode, and disease control (3, 22, 28, 84, 89). However, bacterial wilt control through fumigation or resistant cultivars are not the only options that have been examined. Numerous biological control options have been examined, including non pathogenic strains of bacterial organisms and viruses (bacteriophages) (23, 44, 62, 74). Several organic materials, such as thymol and other crop oils and natural fumigants have

been investigated for control of this pathogen (35, 41, 45, 47, 77, 78). In addition, several cultural practices can alleviate disease levels inflicted by this pathogen. Although research on bacterial wilt control in tomato has examined numerous tactics, crop rotation still remains the most sustainable and effective management practice (49, 55, 69, 86, 93). In areas where crop rotation is not feasible, to date, these other tactics have not been very successful or tested on a commercial level.

Management using Resistant and Grafted Cultivars:

Resistant cultivars have been one of the main areas of focus to combat bacterial wilt of tomato. Resistant tomato cultivars derive their resistance from three major sources. One of these sources, *Solanum pimpenellifolium* Jusl., is assumed to be the source of resistance in the highly resistant Hawaii 7996, 7997, and 7998 breeding lines (34). A second source of resistance originated from a highly resistant landrace, *S. lycopersicum* var. *cerasiforme* (34). The third source of resistance was a cross between *S. pimpinellifolium* and Beltsville #3814 (34). The cultivars Venus, Saturn, and Neptune were the first commercially available cultivars, introduced in the 1990's, to display some level of resistance to bacterial wilt (58, 85). Neptune originated from a heat tolerant breeding line (C1 11d x Campbell 28) crossed with a bacterial wilt resistant breeding line (Hawaii 7997 x Florida 1C). Therefore, the resistance present in Neptune originates from the first source of resistance (*S. pimpenellifolium*) (34, 85). The cultivars BHN 446 and BHN 669 have also been released as bacterial wilt resistant; however, the source of the resistance present in these cultivars is not available due to confidentiality agreements.

Although promising, use of bacterial wilt resistant tomato cultivars has been limited in the United States. Growers have been hesitant to accept these cultivars due to their smaller softer fruit, decreased yields, and less vigorous appearance (77). Louws *et al.* (53) found that

without MBr soil fumigation, yields in bacterial wilt resistant 'BHN 446' and susceptible 'Mountain Spring' cultivars were not significantly different. This yield difference was not due to bacterial wilt control, 'BHN 446' provided significantly higher control of this disease compared to the MBr treatments; however, because of poor agronomic characteristics, the yield of 'BHN 446' was significantly less than the yield from the MBr treated plots (53).

Grafting susceptible cultivars with acceptable agronomic characteristics onto resistant rootstocks has also shown promising results (Fig. 2). Studies in Florida have demonstrated that grafting a susceptible tomato scion Bella Rosa onto the interspecific resistant rootstock Multifort significantly increased the yield of Bella Rosa compared to non-grafted and self-grafted plants (95). Zhao *et al.* (95) also showed that grafted transplants set into non-fumigated soil yielded similarly to non-grafted plants planted in fumigated (MBr:chloropicrin , 50:50) soil. Grafting can be an effective control recommendation for growers with severe soilborne pathogens such as *R. solanacearum* and *Fusarium oxysporum* f.sp. *lycopersici* Sacc., Snyder and Hans. Rivard and Louws (82) in North Carolina conducted a trial to determine the effects of grafting in a field naturally infested with *R. solanacearum*. In trials conducted in 2005 and 2006, they found that the heirloom cultivar German Johnson showed no incidence of bacterial wilt when grafted onto resistant rootstocks of CRA 66 and Hawaii 7996 compared to the non-grafted treatment of German Johnson, which had 75to 79% incidence of bacterial wilt (82). They also reported similar findings with rootstocks resistant to fusarium wilt. Results of the trial indicated that fusarium wilt failed to develop when scions of German Johnson were grafted onto resistant rootstock Maxifort (82). One of the major limitations to using grafting as a control option for any soilborne disease is the cost of incorporating this production practice into commercial programs. In the early to mid 1990's grafting costs for vegetables were estimated at $1.80 to

2.28 per plant compared to the cost of MBr per plant, $0.41 and 0.92 (50). However, recent estimates have dropped considerably since the 1990's; grafted plants for watermelon have been estimated at $1.00 or less in South Texas (50). With the economic costs of grafting becoming more reasonable in price, using grafted plants could prove to be a reliable and effective management technique for disease control.

Figure 2. Grafted seedling.

Management with Cultural Practices:

Cultural practices have been utilized to suppress the severity and spread of bacterial wilt within and throughout fields. The timing of transplanting, drainage, irrigation practices, soil type, crop rotation, and field flooding are all examples of cultural methods examined by various researchers. McCarter *et al*. (59) grew tomato transplants in an infested field and then transported to another field, lacking *R. solanacearum*, at different times in the season. A significantly higher disease incidence was observed in tomato plants that were removed and subsequently transplanted later in the season (May 29 to June 12) than plants that were removed and transplanted earlier (May 13 to May 18) (59). Tomato growers on the ESV use drip tape

irrigation under raised plastic mulched beds. Research by Marouelli *et al*. (55) examined the effects of different irrigation techniques. They tested drip irrigation and overhead sprinkle irrigation on the incidence of tomato bacterial wilt in processing tomato (55). Drip irrigation significantly increased disease incidence when compared to overhead irrigation, 42.5% versus 5.0% respectively (55).

Yuan *et al*. (93) found that fields with deeper ditches, higher beds, and reduced tillage showed lower disease incidence than conventional systems. Fallowing fields has been shown to have some positive effects on suppressing levels of bacterial wilt. Keshwal and Khare (49) showed that a field artificially infested with *R. solanacearum* that was fallowed for a period of 6 and 18 months (one or two summers) showed 100% control of bacterial wilt. Research by Sequeira (86) produced similar results, in that fields fallowed for two years effectively controlled *R. solanacearum* in banana. Although effective results were reported, it is unclear whether or not the fallow fields were bare or weedy which can have an impact on the pathogen populations. Research by Ong *et al.* (72) found that a winter cover crop of vetch reduced disease incidence by 33% and increased the yield of tobacco by 681/kg/ha compared to a bare winter fallow. They also noted that a bare summer or winter fallow did not suppress bacterial wilt populations which contributed to higher bacterial wilt losses (72). This option for control is useful to growers with ample land to utilize crop rotations; however, for growers that have a limited acreage of land, this approach is not feasible, especially for a grower to rotate and still maintain profitable acreages of tomato. Okayama *et al.* (69) examined plots that had been surrounded with concrete frames and were flooded for seven months (September to April) to determine effects on disease incidence in tomato and eggplant. Flooding reduced disease incidence in both crops in soils infested with *R. solanacearum*, but is not often practical (69). In most cases, cultural practices

are adequate for acceptable control of bacterial wilt; however, commercial applications of these tactics are generally not feasible.

Management from Organic Practices:

Several studies have examined bacterial wilt management options for organic growers. For example thymol one of the components found in the oil of thyme, palmarosa oil, and lemongrass oils have been shown to reduce levels of bacterial wilt in tomato (47, 77, 78). When used as a biofumigant, thymol reduced disease incidence to 33.1% and palmarosa oil reduced incidence of disease to 48.1% over the nontreated control (45). In the same study, thymol was evaluated alone and was found to significantly reduce bacterial wilt incidence in the susceptible tomato cultivar Solar Set by 53% (45). In a field study by Bora (7), a mixture of asafetida (cooking spice), turmeric powder, and water (ATW) was applied as a 5% solution to tomato plants as a soil drench at 15, 30, and 45 days after transplanting. Soil drenching with ATW was proven to be the best application method, when applied at the three application intervals, providing the highest level of disease control compared to other soil drenching regimes (7). In the research by Hanudin (35), extracts of garlic (*Allium sativum* L.), shallot (*Allium cepa* L.) and mexican marigold (*Tagetes erecta* L.) were able to suppress bacterial wilt levels on tomato plants inoculated with *R. solanacearum*. Organic soil amendments in the form of fertilizers have been evaluated for control of bacterial wilt. Hernandez and Bustamante (41) tested the use of coffee pulp, sugarcane filter cake, and three types of compost as soil amendments and found that disease severity was less in plots where the compost was added to the soil compared to the other treatments.

Management by Biological Practices:

Research has been conducted examining the potential for microorganisms to suppress *R. solanacearum* populations. The majority of studies have evaluated strains of *Pseudomonas fluorescens* Trevisan, *Bacillus subtilis* Ehrenberg and Cohn and *Pseudomonas aeruginosa* Schroter and Migula for control of bacterial wilt. Minku and Bora (62), treated seedlings with *Ps. fluorescens*, prior to transplanting, reduced disease incidence when applied 14 days before inoculation of *R. solanacearum* compared with other bacterial treatments. In several studies *B. subtilis* reduced disease incidence and severity of *R. solanacearum* in tomato as well. Phae *et al.* (74) demonstrated that a culture suspension of *B. subtilis* (strain NB22) added to *R. solanacearum*-infested soils reduced the incidence of bacterial wilt compared to the control. In another study, Furuya *et al.* (23) found that dip treatments of seedling roots with *Ps. aeruginosa* increased plant survival when tomato plants were transplanted into a field infested with *R. solanacearum*. Jackson and Jones (44) found that 92% of 3-week old tomato transplants treated with bacteriophages prior to inoculation of *R. solanacearum* remained healthy, compared to 5% for the nontreated control.

Management by Induced Resistance:

A pesticide that triggers systemic acquired resistance has been evaluated for control of bacterial wilt in tomato. Growers on the ESV use acibenzolar-*S*-methyl (ASM) (Actigard 50WG, Syngenta Crop Protection, Greensboro, NC) for control of bacterial spot (*Xanthomonas campestris* pv *vesicatoria* (Doidge) Dye) and bacterial speck (*Pseudomonas syringae* pv *tomato* Van Hall). In greenhouse trials with low levels of inoculum, ASM reduced the incidence of bacterial wilt on the susceptible cultivar Solar Set (2). In the field, however, Pradhanang *et al.* (76) found ASM to be ineffective at reducing levels of bacterial wilt in susceptible cultivars, but among moderately resistant cultivars Neptune and BHN-446 ASM reduced disease incidence

levels. They applied the first application two weeks after emergence as a foliar application at a rate of 10 mL (10L/1000 plants), per plant, of ASM solution at 3µg/mL (76). A soil drench application was then applied with 5 mL (5L/ 1000plants) of ASM solution per plant at the same rate as the foliar application (76). They applied 0.03 g of product per 1000 plants in the foliar applications and 0.015g of product per 1000 plants in the soil drench applications. Following transplanting, foliar applications of ASM solution (30µg/mL) were made at 7 day intervals for 30 days, with each plant receiving 30 or 55 mL (358.64 L/ha or 657.51 L/ha) per application depending on the size of the plant (76). After the initial 30 days, plants were treated every 15 days with the same application rates for an additional 30 days (76). In field applications of ASM per plant were applied at a rate of 130.00 g/ha (30ml application) and 238.33 g/ha (55 ml application) depending on the size of the plants. According to the tomato label for bacterial spot and speck, the recommended rates for Actigard 50 WG include an eight week spray program beginning one week after transplanting. The first two applications, weeks one and two, require 23.34 g/ha (one third of an ounce of product per acre) in 286 to 467 L/ha (30 to 50 gallons per acre). Weeks three and four require 35 g/ha (a half ounce of product per acre) applied in 561 to 655 L/ha (60 to 70 gallons per acre). The last four applications, weeks five through eight, require 52.51 g/ha (three quarters of an ounce of product per acre) applied in 655 to 936 L/ha (70 to 100 gallons per acre). Greenhouse applications of ASM are not recommended on the current label for Actigard 50WG. However, a third party label has allowed for greenhouse applications of ASM in Georgia (Dr. Chuck Johnson, Personal communication, 2009). Ji, P. *et al.* (46), in field trials, also found that ASM provided significant protection from bacterial wilt when applied to moderately resistant tomato cultivars inoculated with a high concentration of *R. solanacearum*. However, initial greenhouse applications were made two weeks after seedling

emergence (46). In a study by Araujo *et al.* (5), three tomato cultivars moderately resistant to *R. solanacearum* were transplanted into a field infested with *R. solanacearum* and received 7 weekly applications of ASM by foliar sprays or soil drenches. The three treated cultivars developed wilt symptoms more slowly than the nontreated tomato cultivars when ASM was sprayed onto plants this slower development was observed for the four weeks after transplanting (5).

Management by Chemical Practices:

Despite extensive research examining tomato bacterial wilt control options, most commercial producers still rely upon pre-plant soil fumigation with methyl bromide (MBr) or, in recent years, methyl iodide (MI). The Montreal Protocol and U.S. EPA Clean Air Act have mandated that use of MBr be banned by 2005 (3, 84). Methyl bromide is still used today via Critical Use Exemptions (CUE) granted by the EPA, but its availability is steadily declining and prices of the product are rising. The reduction of MBr availability and future phase out has pushed researchers and producers to search for viable alternatives (84). Methyl bromide is an efficacious broad spectrum biocide and to date there is no single, registered material that provides an equal amount of pest control (14). There have been numerous studies comparing other pesticides with MBr and their ability to control soilborne pathogens, insects, and weeds in various vegetable crops.

Compounds such as 1,3-dichloropropene (1,3-D), metam sodium, metam potassium, dazomet, methyl isothiocyanate generators, and chloropicrin (Chloropicrin 100, Pic Plus Fumigant, Hendrix and Dail, Inc. Greenville, NC) are registered in the US, and have been examined on a worldwide basis. In a study by Gilreath *et al.* (30), 1,3-D + chloropicrin (65:35 v/v; Telone C-35, Dow AgroSciences, Indianapolis, IN) applied as a broadcast treatment

maintained nutsedge and root-knot nematode control and produced tomato yields at the same levels observed in MBr + chloropicrin (67:33) treated plots. Gilreath and Santos (26) found 1,3-D + chloropicrin (83:17 v/v; Telone C-17, Dow AgroSciences, Indianapolis, IN) combined with the herbicide pebulate (Tillam EC, discontinued, Stauffer Chemical Company, Westport, CT) at 4.5 kg/ha produced comparable tomato yields and control of purple nutsedge and fusarium wilt when compared to MBr + chloropicrin (98:2). In another study conducted by Gilreath *et al.* (28), broadcast applications of 1,3-D + chloropicrin (Telone C-35) + in bed applications of chloropicrin, pebulate and trifluralin (Treflan, Dow AgroSciences, Indianapolis, IN) provided an effective wide-spectrum fumigant program, controlling sedges (*Cyperus* spp.) and stubby root nematode (*Trichodorus* spp. Cobb 1913), comparable to MBr + chloropicrin (67:33) in polyethylene-mulched tomato. Both formulations of 1,3-D + chloropicrin (Telone C-35 and Telone C-17) in combination with the herbicides trifluralin, pebulate, and napropamide (Devrinol 50DF, United Phosphorus Inc., King of Prussia, PA) have been shown to be a viable alternative to MBr + chloropicrin (67:33). In another study by Gilreath *et al.* (29), a fumigant combination of 1,3-D + chloropicrin (Telone C-35), pebulate and napropamide applied either in-bed or broadcast over the entire field controlled sedges (*Cyperus* spp. L.), nematodes (*Tylenchorynchus* spp., *Belonolaimus longicaudatus* Rau, 1958 and *Meloidogyne* spp.), and fusarium wilt (*Fusarium oxysporum* f.sp. *lycopersici* Massee) just as well as MBr + chloropicrin (67:33) in tomato. In a study by Csinos *et al.* (14), researchers compared Telone C-17 + metam sodium (Vapam HL, Amvac Chemical Corporation, Newport Beach, CA), Telone C-35 + metam sodium and chloropicrin + metam sodium to MBr + chloropicrin. They compared the compounds using 79 different disease parameters (control of *Rhizoctonia solani* Kuhn, *Pythium* spp., *Fusarium* spp., etc.) resulting in Telone C-17 + metam sodium performing better than the

other treatments and was not significantly different in 5 of the 79 parameters; however, the combination was superior to MBr + chloropicrin (98:2) in 3 of the 79 parameters and inferior in only 2 (14).

Several experimental products have shown promise as MBr alternatives, but their chemistry, toxicology, and environmental fate have not been fully investigated. Two compounds receiving consideration are, propargyl bromide (Dow Chemical Company, Midland, MI), and dimethyl disulfide (DMDS) (Paladin, United Phosphorus Inc., King of Prussia, PA) (84). Propargyl bromide has been tested in Florida, examining application rates of 45 to 224 kg/ha, with rates from 45 to 112 kg/ha providing the best control of root-knot nematodes, fusarium wilt, *Phytophthora capsici* Leonian and yellow nutsedge (*Cyperus esculentus* L.) (65, 66, 84). Subbarao (89) found that MI and propargyl bromide were comparable to MBr for control of pathogens and weeds.

A promising MBr alternative that has been implemented by growers on the ESV is MI (Midas, Arysta LifeScience North America, LLC, Cary, NC). Gilreath and Santos (27) found that nutsedge control was best achieved at a rate of 392 kg/ha using a 50/50 formulation of MI:chloropicrin (84). Methyl iodide has not been linked to ozone depletion, and was found to parallel MBr in controlling *Phytophthora citricola* Sawada, *P. cinnamomi* Rands., *P. parasitica* Dastur, *Rhizoctonia solani* Kuhn, the nematode *Heterodera schachtii* Schmidt, 1871, and weeds such as purple nutsedge (*Cyperus rotundus* L.), annual bluegrass (*Poa annua* L.), purslane (*Portulaca oleracea* L.), and tall mustard (*Sisymbrium irio* L.) (68, 80). Researchers in Florida conducted fumigant trials to compare MI and MBr at controlling weed seed in strawberry nurseries (22). Results from the trials conducted showed no significant differences between

either fumigant for the control of prostrate knotweed, common purslane and common chickweed, with over 94% control (22).

Another product receiving consideration as an alternative to MBr is DMDS. Two trials were conducted in Florida on cut flowers, the first of which demonstrated DMDS to control weeds as effectively as MBr (84). The second trial yielded comparable results to MBr in controlling Pythium root rot, root-knot nematode juveniles, and maintained equivalent marketable yields while reducing the vegetative growth of cockscomb (*Celosia argenta* var. *crista* L.) (11, 84). Research in Georgia in the spring of 2008 reported DMDS to provide significant control of large crabgrass (*Digitaria sanguinalis* L.) and purple nutsedge compared to the non-treated control (15). Nursery fumigation trials on strawberry in Spain in 2007, found no significant differences in control of nightshades (*Solanum* spp.), common purslane or common lambsquarters (*Chenopodium album* L.) between DMDS (400:150 kg/ha v:v DMDS and chloropicrin) and MBr (300 kg/ha, 50:50 MBr and chloropicrin) (25). Research on strawberry in Spain in 2007 and 2008 compared MBr alternatives for control of the root lesion nematode (*Pratylenchus penetrans* (Cobb, 1917) Chitwood & Oteifa, 1952). Results from these trials indicated DMDS (300:100 kg/ha of DMDS and chloropicrin) and MBr + chloropicrin (400 kg/ha of MBr and Chloropicrin, 50:50 v:v) were not significantly different in control of the nematode (52). Average yields also showed no significant differences between DMDS and MBr + chloropicrin (52).

Most of the literature presented focuses on possible methyl bromide alternatives that could potentially control *R. solanacearum*. This research examined the potential control recommendations and how well they controlled similar diseases such as fusarium wilt as well as some others. However, some of the more promising control recommendations have been

evaluated for control of *R. soalanacearum*. Research by Olsen and Rich was conducted on tomato comparing MBr + chloropicrin (67:33) and DMDS + chloropicrin (79:21) at numerous rates (71). They compared the fumigants for the control of bacterial wilt, nutsedge, and total yield (71). The results from the 2007 trial indicated no significant differences between MBr or DMDS for control of bacterial wilt, nutsedge and total yield (71). Research in Florida showed that plots treated with MI + chloropicrin (50:50) were not significantly different than plots treated with MBr + chloropicrin (67:33) in control of *R. solanacearum* and yield (70).

Management by Water Sanitation:

Research has been conducted examining different methods of sanitizing irrigation water as well as sanitizing and purifying waste water. Hong *et al.* proposed examining chlorine and peroxide as possible chemical treatments to eradicate *R. solanacearum* from irrigation water (42). Greenhouse trials conducted by Berenguer and colleagues (6) showed sodium hypochlorite to significantly reduce levels of *Pythium* and *Phytophthora* spp. in irrigation water compared to ultraviolet (UV) radiation and the non-treated control. Although UV radiation was not as effective as sodium hypochlorite it was still significantly more effective than the non-treated control (6). Research by Gomila *et al.* (32) showed UV radiation to successfully remove bacteria (total colliforms, fecal colliforms, *Escherichia coli*, enterococci, and spores of sulphite-reducing clostridia) and viruses (enteroviruses, somatic coliphages, F-specific coliphages, and phages infecting *Bacteroides fragilis* Castellani and Chalmers and *Bacteroides thetaiotaomicron* Castellani and Chalmers from waste water. Illueca-Munoz *et al.* (43), showed 16 J/m^2 UV radiation reduced fecal colliforms in waste water from 70,000 cfu per 100 mL to 5 cfu per 100 mL. Research in the Netherlands showed exposing irrigation water to 95 ^oC for thirty seconds was successful at killing one hundred percent of all bacteria, fungi, viruses and nematodes

present (6). To date, water sanitation has not been evaluated as a possible management strategy for suppressing tomato bacterial wilt.

Bacterial wilt is a very aggressive soilborne pathogen, especially on tomato. The more information tomato growers are provided about the management and biology of this pathogen, the better equipped they will be to reduce the threat of bacterial wilt epidemics. Currently there are few proven management tactics for control of *R. solanacearum* in tomato. Further research on the biology of *R. solanacearum*, as well as alternative control methods combining soil fumigants and cultural practices, needs to be conducted. The ESV is a productive region for fresh market tomato growers; the lack of land available for rotation forces the continuous planting of tomato in the same fields year after year. Under these monoculture conditions, the need for information and research is imperative for the sustainability of tomato production on the ESV.

Research Objectives:

1. To determine the temporal and spatial movement of *R. solanacearum* throughout tomato fields.

2. To investigate existing and newly released tomato cultivars in combination with acibenzolar-S-methyl to suppress bacterial wilt.

Literature Cited:

1. Akiew, E. B. and Hams, F. 1990. *Archontophoenix alexandrae*, a new host of *Pseudomonas solanacearum* in Australia. Plant Disease 74: 615.
2. Anith, K.N., M.T. Momol, J.W. Kloepper, J.J. Marois, S.M. Olson, and J.B. Jones. 2004. Efficacy of plant growth-promoting rhizobacteria, acibenzolar-*S*-methyl, and soil amendment for integrated management of bacterial wilt on tomato. Plant Disease 88:669-673.
3. Anonymous. 1998. Montreal Protocol on substances that deplete the ozone layer. Online. United Nations Environ. Prog. (UNEP). Methyl Bromide Tech. Options Comm., 1998 Assess. Altern. Methyl Bromide.
4. Aragaki, A. and Potts, M.J. 1965. Bacterial wilt of ornamental gingers (*Hedychium* spp.) caused by *Pseudomonas solanacearum*. Plant Disease. Rep. 49: 378-79.
5. Araujo, J.S. de P., Goncalves, K.S., Ribeiro, R. de L.D., Polidoro, J.C. and Rodrigues, R. 2005. Resistance to tomato bacterial wilt induced by acibenzolar–S–methyl. Acta Horticulturae 695: 429-34.
6. Berenguer, J. J., Escobar, I., and Garcia, M. 2001. Methods to control *Pythium* and *Phythophthora* in cold plastic houses. Acta Horticulturae 559:759-763.
7. Bora, L.C. 1995. Management of bacterial wilt of tomato by organic formulations. Plant Health 1: 74-6.
8. Buddenhagen, I. W. 1986. Bacterial wilt revisited. ACIAR Proc. 13 pp 126-43.
9. Buddenhagen, I. W. and Kelman, A. 1964. Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum*. Annual Review. Phytopathology 2: 203-30.

10. Buddenhagen, Ivan, Sequeira, Louis, and Kelman, Arthur. 1962. Designation of races in *Pseudomonas solanacearum*. Phytopathology 52:726.

11. Church, G., Rosskopf, E. and Holizinger, J. 2004. Evaluation of DMDS for production of ornamental cockscomb (*Celosia agentea*). Annu. Int. Res. Conf. on Methyl Bromide Alternatives and Emissions Reductions, MBAO, pp 87-1-87-5.

12. Ciampi, L. and Sequeira, L. 1980. Multiplication of *Pseudomonas solanacearum* in resistant potato plants and the establishment of latent infections. American Potato Journal 57: 319-29.

13. Ciampi, L., Sequeira, L. and French, E. R. 1980. Latent infection of potato tubers by *Pseudomonas solanacearum*. American Potato Journal 57: 377-86.

14. Csinos, A.S., Sumner, D.R., Johnson, W.C., Johnson, A.W., McPherson, R.M. and Dowler, C.C. 2000. Methyl bromide alternatives in tobacco, tomato, and pepper transplant production. Crop Protection 19: 39-49.

15. Culpepper, S.,Sosnoskie, L., Rucker, K.,Tankersley, B. and Langston, D. DMDS or the 3-Way: which is more effective in Georgia?. 2008 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions.

16. Denny, T. P. 2005. A short history of the biochemical and genetic research on *Ralstonia solanacearum* pathogenesis. Pages 323-334 in: Bacterial Wilt Disease and the *Ralstonia solanacearum* Species Complex. Allen, C., Prior, Ph., and Hayward, A.C. eds. The American Phytopathological Society, St. Paul, Minnesota.

17. Dianese, J. C., Dristig, M. C. G. and Cruz, A. P. 1990. Susceptibility to wilt associated with *Pseudomonas solanacearum* among six specieas of Eucalyptus growing in equatorial Brazil. Australas. Plant Pathology 19: 71-6.

18. Dukes, P. D., Morton, D. J. and Jenkins, S. F. Jr. 1965. Infection of indigenous hosts by *Pseudomonas solanacearum* in south Georgia. Phytopathology 55: 1055 (Abstract).

19. Elphinstone, J. G., Stanford, H. M., and Stead, D. E. 1997. Detection of *Ralstonia solanacearum* in potato tubers, *Solanum dulcamara* and associated irrigation water. Pages 133-139 in: Bacterial Wilt Disease: Molecular and Ecological Aspects. Prior, Ph., Allen, C., and Elphinstone, J., eds. Springer-Verlag Berlin Heidelberg New York.

20. Fegan, M. and Prior, P. 2005. How complex is the "*Ralstonia solanacearum* species complex"?. Pages 449-461 in: Bacterial wilt disease and the *Ralstonia solanacearum* species complex. Allen, C., Prior, P. and Hayward, A. C., eds. American Phytopathological Society (APS Press) St. Paul.

21. Fegan, M., Taghavi, M., Sly, L. I., and Hayward, A. C. 1997. Phylogeny, Diversity and Molecular Diagnostics of *Ralstonia solanacearum*. Pages 19-33 in: Bacterial Wilt Disease: Molecular and Ecological Aspects. Prior, Ph., Allen, C., and Elphinstone, J., eds. Springer-Verlag Berlin Heidelberg New York.

22. Fennimore, S. A., Haar, M. J., Goodhue, R. E., Winterbottom, C. Q. 2008. Weed Control in Strawberry Runner Plant Nurseries with Methyl Bromide Alternative Fumigants. Hortscience 43(5):1495–1500.

23. Furuya, N., Yamasaki, S., Nishioka, M, Shiraishi, I., Iiyama, K. and Matsyama, N. 1997. Antimicrobial activities of *pseudomonads* against plant pathogenic organisms and efficacy of *Pseudomonas aeruginosa* ATCC7700 against bacterial wilt of tomato. Annals of the Phytopathological Society of Japan 63: 417-24.

24. Gallegly, M.E. and Walker, J.C. 1949. Relation of Environmental Factors to bacterial wilt of tomato. Phytopathology 39: 936-46.

25. García-Sinovas, D., García-Méndez, E., Andrade, M. A., Becerril, M. De Cal, A., Melgarejo, P., Salto, T., Martínez-Beringola, M. L., Redondo, C., Martínez-Treceño, A., Medina, J. J., Soria, C., and López-Aranda, J. M. Strawberry nurseries in spain: alternatives to mb, 2007 results. 2008 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions.

26. Gilreath, James P. and Santos, B. M. 2004. Methyl bromide alternatives for weed and soilborne disease management in tomato (*Solanum lycopersicum*). Crop Protection 23: 1193-98.

27. Gilreath, J. P., and Santos, B. M. 2004. Purple nutsedge control with iodomethane. Annu. Int. Res. Conf. on Methyl Bromide Alternatives and Emissions Reductions, MBAO, pp 51-1-51-2.

28. Gilreath, J. P., Santos, B.M., Busacca, J.D., Eger, J. E. Jr., Mirusso, J.M. and Gilreath, P.R. 2006. Validating broadcast application of Telone C-35 complemented with chloropicrin and herbicides in commercial tomato farms. Crop Protection 25: 79-82.

29. Gilreath, J.P., Santos, B.M., Gilreath, P.R., Jones, J.P. and Noling, J.W. 2004. Efficacy of 1,3-dichloropropene plus chloropicrin application methods in combination with pebulate and napropamide in tomato. Crop Protection 23: 1187-91.

30. Gilreath, J.P., Santos, B.M., Gilreath, P.R., and Noling, J.W. 2005. Validation of 1,3-dichloropropene plus chloropicrin broadcast application in tomato grower fields. Journal of Vegetable Science 11: 133-39.

31. Gionson-Monsalud,R., Aspiras, R. B., Barraquio, W. L., Manguiat, I. J., and Natural, M. P. 2002. Population changes of *gusA*-marked *Ralstonia solanacearum* in the soil under two moisture and temperature conditions. The Phillipine Agricultural Scientist 85:161-169.

32. Gomila, Margarita, Solis, Javier J., David, Zoyla, Ramon, Cristina, and Lalucat, Jorge. 2008. Comparative reductions of bacterial indicators, bacteriophage-infecting enteric bacteria and enteroviruses in wastewater tertiary treatments by lagooning and UV-radiation. Water Science and Technology 58:2223-2233.

33. Granada, G. A. 1988. Latent infections induced by *Pseudomonas solanacearum* in potato and symptomless plants. Report of the planning conference on bacterial diseases of the potato. pp 93-107.

34. Hanson, P.M., Licardo, O., Hanudin, Wang, J. F., and Chen, J.-T. 1998. Diallel analysis of bacterial wilt resistance in tomato derived from different sources. Plant Dis. 82:74-78.

35. Hanudin. 1987. Controlling the incidence of the bacterial wilt (*Pseudomonas solanacearum* E.F. Smith) on tomato plants by some plant extracts. Buletin Penelitian Hortikultura 15: 60-6.

36. Hayward, A.C. 1994. The Hosts of Pseudomonas solanacearum. p. 9-24. In: Bacterial wilt: the disease and its causative agent, *Pseudomonas solanacearum*. Hayward, A.C., and Hartman, G.L., eds. Wallingford (United Kingdom), Cab International.

37. Hayward, A.C. 1994. Systematics and Phylogeny of *Pseudomonas solanacearum* and

Related Bacteria. p. 123-135. In: Bacterial wilt: the disease and its causative agent, *Pseudomonas solanacearum*. Hayward, A.C., and Hartman, G.L., eds. Wallingford (United Kingdom), Cab International.

38. Hayward, A.C. 1991. Biology and Epidemiology of Bacterial Wilt caused by *Pseudomonas solanacearum*. Annual Review of Phytopathology 29: 65-87.

39. Hayword, A. C. 1964. Characteristics of *Pseudomonas solanacearum*. Journal of Applied Bacteriology 27: 265-77.

40. He, L. Y., Sequeira, L. and Kelman, A. 1983. Characteristics of strains of *Pseudomonas solanacearum* from China. Plant Disease 67: 1357-61.

41. Hernandez Garboza, L. and Bustamante Rojas, E. 2001. Biological control of bacterial wilt in tomato with organic amendments. Manejo Integrado de Plagas 2: 18-28.

42. Hong, J., Ji, P., Momol, M. T., Jones, J. B., Olson, S. M., Pradhanang, P., and Guven, K. 2005. *Ralstonia solanacearum* detection in tomato irrigation ponds and weeds. Proc. Ist IS on tomato diseases. Acta Horticulturae 695:309-311.

43. Illueca-Munoz, J., Mendoza-Roca, J. A., Iborra-Clar, A., Bes-Pia, A., Fajardo-Montanana, V., Martinez-Francisco, F. J., and Bernacer-Bonora, I. 2008. Study of different alternatives of tertiary treatments for wastewater reclamation to optimize the water quality for irrigation reuse. Desalination 222:222-229.

44. Jackson, L.E. and Jones, J.B. 2005. Bacteriophage: a viable bacteria control solution. Acta Horticulturae 695: 109-17.

45. Jatala, P., Martin, C. and Mendoza, H. A. 1988. Role of nematodes in disease

expression by *Pseudomonas solanacearum* and strategies for screening and breeding for combined resistance. Report of the planning conference on bacterial diseases of the potato. pp 35-37.

46. Ji, P, Momol, M.T., Olson, S.M., Hong, J., Pradhanang, P., Anith, K.N. and Jones, J.B. 2005. New tactics for bacterial wilt management on tomatoes in the Southern U.S. Acta Horticulturae 695: 153-59.

47. Ji, P., Momol, M.T., Olson, S.M., Pradhanang, P.M. and Jones, J.B. 2005. Evaluation of thymol as a biofumigant for control of bacterial wilt of tomato under field conditions. Plant Disease 89:5 497-500.

48. Kelman, A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum*. NC Agricultural Experiment Station. Technical Bulletin 99: 194.

49. Keshwal, R.L. and Khare, U. K. 2000. Effect of fallowing on incidence of bacterial wilt of tomato in different soil types. Agricultural Research Communication Centre, Karnal, India, Bhartiya Krishi Anusandhan Patrika. 15: 60-4.

50. King, S. R., Davis, A. R., Liu, W., and Levi, A. 2008. Grafting for Disease Resistance. Hortscience 43(6):1673-1676.

51. Krauz, J. P. and Thurston, H. D. 1975. Breakdown of resistance to *Pseudomonassolanacearum* in tomato. Phytopathology 65: 1272-74.

52. López-Aranda, J. M., Miranda, L., Soria, C., Domínguez, P., Pérez-Jiménez, R. M., Martín-Sánchez, P. M., Talavera, M., Romero, F., De Los Santos, B., Medina, J. J. Strawberry production in spain: alternatives to mb, 2008 results. 2008 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions.

53. Louws, F.J., J.G. Driver, L.M. Blackwell, and W.C. Batten. 2002. Evaluation of host resistance, plant activators, biologicals, and fumigants to manage tomato bacterial wilt, 2001. Biological and Cultural Tests 18:PT016.

54. Mao, G. Z., and He, L. Y. 1997. Relationship of wild type strain motility and interaction with host plants in *Ralstonia solanacearum*. Pages 184-191 in: Bacterial Wilt Disease: Molecular and Ecological Aspects. Prior, Ph., Allen, C., and Elphinstone, J., eds. Springer-Verlag Berlin Heidelberg New York.

55. Marouelli, W.A., Lopes, C.A. and Silva, W.L.C. 2005. Incidence of bacterial wilt on processing tomato under drip and sprinkle irrigation. Horticultura Brasileira 23:2 320-333.

56. Martin, C. and Nydegger, U. 1982. Susceptibility of *Cyphomandra betacea* to *Pseudomonas solanacearum*. Plant Disease 66: 1025-27.

57. Mayers, P. E. and Hutton, D. G. 1987. Bacterial wilt a new disease of custard apple: symptoms and etiology. Ann. Appl. Biol. 111: 135-41.

58. McCarter, S.M. 1993. Bacterial Wilt. p. 28-29. in: Compendium of Tomato Diseases. J.B. Jones, J.P. Jones, R.E. Stall, and T.A. Zitter, eds. American Phytopathological Society, St. Paul, MN. 73 pp.

59. McCarter, S. M., Barksdale, T.H. and Jaworski, C.A.. 1971. Reduction of bacterial wilt by early harvest of Tomato transplants. Phytopathology 61: 849-851.

60. Mew, T. W. and Ho, W. C. 1977. Effect of soil temperature on resistance of tomato cultivars to bacterial wilt. Phytopathology 67: 909-11.

61. Middleton, K. J. and Hayward, A. C. 1990. Bacterial Wilt of Groundnuts. Proc.

ACIAR/ICRISAT collaborative res. plan. meet., Genting Highlands, Malaysia, 18-19 March. ACIAR Proc. 31.

62. Minku, D. and Bora, L.C. 2000. Biological control of bacterial wilt of tomato caused by *Ralstonia solanacearum*. Journal of the Agricultural Science Society of North-East India 13: 52-5.

63. Napiere, C.M. and Quimio, A.J. 1980. Influence of root-knot nematode on bacterial wilt severity in tomato. Annals of Tropical Research 2: 29-39.

64. National Agricultural Statistics Service (NASS). 2009. United States Department of Agriculture. December 11, 2009. http://www.nass.usda.gov/Data_and_Statistics/index.asp.

65. Noling, J. W., Gilreath, J. P., and Rosskopf, E. N. 2001. Alternatives to methyl bromide field research effort for nematode control in Florida. Annu. Int. Res. Conf. on Methyl Bromide Alternatives and Emissions Reductions, MBAO, pp 14-1-14-3.

66. Noling, J. W., Rosskopf, E. N., and Chellemi, D. O. 2002. Impacts of alternative fumigants on soil pest control and tomato yield. Annu. Int. Res. Conf. on Methyl Bromide Alternatives and Emissions Reductions, MBAO, pp 30-130-3.

67. Nyangeri, J. B., Gathuru, E. M. and Mukunya, D. M. 1984. Effect of latent infection on the spread of bacterial wilt of potatoes in Kenya. Tropical Pest Management 30: 163-5.

68. Ohr, H.D., Sims, J.J., Grech, N.M., Becker, J.O., and McGiffen, M.E., Jr. 1996. Methyl iodide, an ozone-safe alternative to methyl bromide as a soil fumigant.

69. Okayama, K., Sugimura, T., Matsutani, S. and Nishizaki, M. 2003. Effects of block

rotation, rootstocks, and flooding in fields on prevention of bacterial wilt in eggplant. Bulletin of the Nara prefectural Agricultural Experiment Station 34: 43-50.

70. Olson, S. M. 2007. Efficacy of Midas (50/50) as a soil fumigant for tomato production. Annu. Int. Res. Conf. on Methyl Bromide Alternatives and Emissions Reductions, MBAO, pp 32-1:32-4.

71. Olson, S. M., and Rich, J. 2007. Efficacy of Paladin (DMDS) as a soil fumigant for tomato and cantaloupe production. Annu. Int. Res. Conf. on Methyl Bromide Alternatives and Emissions Reductions, MBAO, pp 68-1:68-4.

72. Ong, K. L., Fortnum, B. A., Kluepfel, D. A., and Riley, M. B. 2007. Winter cover crops reduce bacterial wilt of flue-cured tobacco. Online. Plant Health Progress doi:10.1094/PHP-2007-0522-01-RS.

73. Persley, G. J. 1986. Bacterial wilt disease in Asia and the South Pacific. Proc. Int. Workshop, PCARRD, Los Banos, Philipines, 8-10 Oct. 1985. ACIAR Proc. 13. 145 pp.

74. Phae, C.G., Shoda, M., Kita, N., Nakano, M. and Ushiyama, K. 1992. Biological control of crown and root rot and bacterial wilt of tomato by *Bacillus subtillus* NB22. Annals of the Phytopathological Society of Japan 58: 329-39.

75. Power, R. H. 1983. Relationship between the soil environment and tomato resistance to bacterial wilt (*Pseudomonas solanacearum*) 4. Control Methods. *Surinaamse Landbouw* 31: 39-47.

76. Pradhanang, P.M., Ji, P, Momol, M.T., Olson, S.M., Mayfield, J.L. and Jones, J.B. 2005.

Application of acibenzolar –S– methyl enhances host resistance in tomato against *Ralstonia solanacearum*. Plant Disease 89: 989-993.

77. Pradhanang, P.M., Momol, M.T., Olson, S.M. and Jones, J.B. 2003. Effects of plant essential oils on *Ralstonia solanacearum* population density and bacterial wilt incidence in tomato. Plant Disease 87: 423-27.

78. Pradhanang, P.M., Momol, M.T., Olson, S.M. and Jones, J.B. 2005. Management of bacterial wilt in tomato with essential oils and systemic acquired resistance inducers. Bacterial wilt disease and the *Ralstonia solanacearum* species complex. pp. 133-38.

79. Quinon, V. L., Aragaki, M. and Ishii, M. 1964. Pathogenicity and serological relationships of three strains of *Pseudomonas solanacearum* in Hawaii. Phytopathology 54: 1096-99.

80. Reddy, P. P., Singh, D. B. and Kishun, R. 1979. Effect of root-knot nematode on the susceptibility of Pusa Purple Cluster Brinjal to bacterial wilt. Curr. Sci. 48: 915-16.

81. Ristaino, J. B. and Thomas, W. 1997. Agriculture, methyl bromide, and the ozone hole: can we fill the gaps?. Plant Disease 81: 964-77.

82. Rivard, C. L., and Louws, F. J. 2008. Grafting to Manage Soilborne Diseases in Heirloom Tomato Production. Hortscience 43(7):2104–2111.

83. Robertson, A. E. 1997. Factors affecting the population of *Ralstonia solanacearum* in a naturally infested field planted in tobacco. Pages 369-375 in: Bacterial Wilt Disease: Molecular and Ecological Aspects. Prior, Ph., Allen, C., and Elphinstone, J., eds. Springer-Verlag Berlin Heidelberg New York.

84. Rosskopf, E.N., Chellemi, D.O. Kokalis-Burelle, N. and Church, G.T. 2005. Alternatives to methyl bromide: A Florida perspective. Online. Plant Health Progess.

85. Scott, J.W., J.B. Jones, and G.C. Somodi. 1995. 'Neptune', a heat–tolerant, bacterial–wilt tolerant tomato. HortScience 30:641-642.

86. Sequeira, L. 1962. Control of bacterial wilt of banana by crop rotation and fallowing. Tropical Agriculture (Trinidad) 39: 211-17.

87. Shiomi, T., Mulya, K. and Verma, R. K. 1989. Bacterial wilt of cashew (*Anacardium occidentale*) caused by *Pseudomonas solanacearum* in Indonesia. Ind. Crops Res. Journal 2: 29-35.

88. Suatmadji, R. W. 1986. Complex diseases involving nematodes and *Pseudomonas solanacearum* in potatoes in the tropics and subtropics. ACIAR Proc. 13. pp 120-125.

89. Subbarao, Krishna V. 2002. Methyl bromide alternatives: meeting the deadlines. Phytopathology 92:1334-1336.

90. Sunaina, V., Kishore, V. and Shekhawat, G. S. 1989. Latent survival of *Pseudomonas solanacearum* in potato tubers and weeds. Pflanzenkr. Pflanzenschutz 96: 361-64.

91. Tsuchiya, K. and Horita, M. 1997. Genetic Diversity of *Ralstonia solanacearum* in Japan. Pages 61-73 in: Bacterial Wilt Disease: Molecular and Ecological Aspects. Prior, Ph., Allen, C., and Elphinstone, J., eds. Springer-Verlag Berlin Heidelberg New York.

92. Tung, P. X., Rasco, E. T. Jr., Vander Zaag, P. and Schmiediche, P. 1990. Resistance to

Pseudomonas solanacearum in the potato. II. Aspects of host-pathogen-environment interaction. Euphytica 45: 211-15.

93. Yuan, L.H., Su, Q.L. and Yi, Y.H. 1992. A brief report on the technique for control of tomato bacterial wilt disease. Plant Protection 18: 48-9.

94. Zehr, E. T. 1969. Studies of the distribution and economic importance of *Pseudomonas solanacearum* E. F. Smith in certain crops in the Philippines. Philipp. Agric. 53: 218-23.

95. Zhao, X., Simonne, E. H., Hochmuth, R. C. Grafting as an Alternative to Methyl Bromide in Field Tomato Production. Oral Session Abstracts. 106th Annual International Conference of the American Society for Horticultural Science, 2009.

Table 1.*Ralstonia solanacearum* classified by race based on host range.

Race	Host(s)
1	tobacco, tomato, solanaceous sp., some ornamentals
2	triploid bananas
3	tomato and potato
4	ginger (edible)
5	Mulberry

Table 2. Differentiation of biovars of *Ralstonia* (*Pseudomonas*) *solanacearum*.

Property		Biovar 1	Biovar 2	Biovar 3	Biovar 4	Biovar 5
Utilization of dissacharides	Mannitol	-	-	+	+	+
	Sorbitol	-	-	+	+	-
	Dulcitol	-	-	+	+	-
	Trehalose	+	-[z]	+[y]	+[y]	+
Oxidation of hexose alcohols	Lactose	-	+	+	-	+
	Maltose	-	+	+	-	+
	Cellobiose	-	+	+	-	+
Gas from nitrate	denitrification	-[z]	-[z]	+	+	+

[z] Uncommon isolates give a positive reaction
[y] Uncommon isolates give a negative reaction

Table 3. *Ralstonia solanacearum* biovars classified by phylotype based on geographical origin.

Phylotype	Biovars	Origin
I	3, 4, 5	Asia
II	1, 2, 2T	USA
III	1, 2T	Africa
IV	1, 2, 2T	Indonesia, Australia and Japan

Chapter 2

Temporal and spatial distribution of tomato bacterial wilt on Virginia's Eastern Shore

Introduction:

More than 4,500 acres of fresh market tomato (*Solanum lycopersicum* L.) are grown annually on the Eastern Shore of Virginia (ESV) (9). Bacterial wilt of tomato is caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al.*, and is one of the most problematic diseases for tomato growers on the ESV. Annual losses incurred by growers have not been reported, but in certain fields, yield reductions of 50% have been observed. Traditionally, crop rotation with non-susceptible crops, such as cereals, has been the most effective control recommendation in areas where the disease is present; however, ESV tomato producers lack the available farm land to viably rotate crops for an extended period of time and maintain profitability. Therefore improved disease management tactics are needed to sustain tomato production on the ESV. .

Ralstonia solanacearum is a gram negative bacterium with 1-4 polar flagella; it is aerobic, catalase and oxidase positive, and converts nitrates to nitrites (8). The pathogen can survive over a wide temperature range from 10 to 41 ^{o}C, with an optimum between 25 and 35 ^{o}C (4, 8). Infection by this pathogen occurs through wounding or natural openings in the root tissue of susceptible hosts (4, 5, 8). The pathogen can infect over 200 plant species such as tomato, potato (*Solanum tuberosum* L.), banana (*Musa acuminata* Colla.), tobacco (*Nicotiana tabacum* L.), and many weed species that are asymptomatic (4). Although considerable research has been conducted on the biology of the pathogen, such as its host range, disease cycle, and control; this disease remains a problem on tomato in Virginia and the southeast. The temporal and spatial spread of bacterial wilt within commercial tomato fields over time has not been thoroughly investigated. In a study by Madden *et al.* (6), ordinary runs analysis was used to analyze the

distribution pattern of *Maize dwarf mosaic virus* in sweet corn (*Zea mays* L.). They noted the usefulness of this method in evaluating temporal and spatial disease spread. Understanding the distribution of bacterial wilt within commercial fields may lead to better management techniques and strategies.

The objective of this research was to determine the temporal and spatial dynamics of bacterial wilt within commercial tomato fields on the ESV. Ordinary runs analysis was used to determine distribution patterns of bacterial wilt in multiple commercial tomato fields and growing seasons in Virginia. Understanding distribution patterns of this disease can provide insight into its spread, hopefully leading to reductions in disease within infested fields.

Materials and Methods:

Field Trial Agronomic Information. Four ESV tomato fields across three growing seasons were observed to document the temporal and spatial distribution of tomato bacterial wilt in fields naturally infested with *R. solanacearum*. All observations were conducted in fields containing Bojac sandy loam soils. Standard production practices for plasticulture production of fresh market tomato were followed in all trials (2). Black polyethylene mulch was placed over raised beds with drip tape irrigation tubing laid within the beds in all trials conducted in Painter in 2006, 2007, and 2008. Metalized mulch was placed over raised beds with drip tape irrigation tubing laid within the beds in the Machipongo, Va trial conducted in 2007. The cultivar BHN602 was used in the trials conducted in Painter and Machipongo for all years. Fertilization by the grower occurred according to local recommendations (2). The plants were staked and strung throughout the growing season using the string-weave method. Insect, foliar disease and weed control was accomplished using standard production practices for the ESV.

Bacterial Wilt Incidence Assessments. For each field trial, four replicates were established with each replicate consisting of twenty rows with one hundred plants per row. The number and position of plants in each row exhibiting symptoms of bacterial wilt infection was recorded every 7 to 10 days. In 2006, the first trial was established in a commercial tomato field near Painter with initial observations on July 14 and continuing every 7 to 10 days until August 14, 2006. Two locations were established in 2007, the same field near Painter as the 2006 trial and a field near Machipongo. The field in Painter was observed from June 14 to August 1, 2007. The field in Machipongo was assessed from June 21 to July 25, 2007. A single trial was conducted near Painter in 2008 at a different field than that assessed in 2006 and 2007. Observations began June 20 and ended August 5, 2008. Trials were stopped after producers finished harvesting each field.

Data analysis. Disease observations from all trials were analyzed using ordinary runs analysis (3). Individual disease assessments were entered into grid sheets in Microsoft Excel (2003, Microsoft Corporation, Redmond, WA) and calculations were performed to determine which rows exhibited clustering. Analyses were performed both within rows and across rows to determine if there were significant patterns of clustering. Rows exhibiting a clustered distribution were divided by the total number of rows in that particular repetition and assessment date to determine the percentage of rows showing a clustered distribution. There are three main parts to the equation as explained by Madden *et al.* (6):

1. $E(U)= 1 + 2m(N-m)/N$

Expected number of runs= 1+2(number of infected plants per row)(number of non-infected plants per row)/total number of plants per row

In this part of the equation m is equal to the number of infected plants in a row and N is equal to the total number of plants. The total number of runs is represented by U where the observed number of runs will be less than E(U) if there is clustering of infected plants.

2. $^{S}u=(2m(N-m)[2m(N-m)-N]/[N^{2}(N-1])^{1/2}$

The standard deviation of U is given in this part of the equation.

3. $Z_u = [U + 0.5 – E(U)]/^{S}u$

This is the equation for a standardized U and the 0.5 constant is the correction for continuity. The sampling distribution of Z_u is the standard normal distribution and if the value is a large negative number (<-1.64) the distribution is considered to be clustered.

Agricultural Research Manager, Version 7 (ARM 7) (Gylling Data Management, Inc. Brookings, South Dakota) was used to determine if a positive correlation existed between the disease incidence and the percentage of rows showing a clustered distribution. In addition ARM 7 was used to conduct analyses of variance to determine if there were significant differences between the assessment dates within each trial for disease incidence and the percentage of rows showing a clustered distribution of disease. Means were compared using Fisher's LSD with a p-value of 0.05 indicating significance.

Results:

2006 Painter Trial. As the season progressed the percentage of rows exhibiting a clustered distribution of diseased plants increased (Fig. 1). At the conclusion of this trial, over 90% of the rows within each replication showed a significant clustered pattern of wilted plants within rows. There was a positive correlation between disease incidence and rows showing a clustered distribution (Table 1). Analysis of variance for disease incidence across assessment dates indicated a significant increase in disease incrementally over all four observational dates

(Table 2). The percentage of rows with a clustered distribution increased significantly from the first assessment date (July 14) to the latter assessment dates (July 26, August 3, and August 14), however the latter three dates were not significantly different from one another.

2007 Painter Trial. As in 2006, the percentage of rows exhibiting a clustered distribution within each replication increased for both trials as the 2007 season progressed. In the trial conducted in Painter (Fig. 2) at least 95 percent of the rows showed significant clustering of diseased plants within rows by the end of the trial (Aug. 1). A positive correlation between disease incidence and the percentage of clustered rows existed for the 2007 trial in Painter (Table 1). Similar differences were detected in the percentage of rows exhibiting a clustered distribution of disease, with most of the dates significantly different from one another. As the season progressed significant increases in disease were observed for the five assessment dates between July 3 to August 1 (Table 2).

2007 Machipongo Trial. Similar results were observed in the Machipongo trial (Fig. 3) as in the 2006 and 2007 Painter trials. As the season progressed, the percentage of clustered rows increased over time. By the end of the season (July 25), replications 2, 3, and 4 had at least 90 percent of the rows exhibiting significant clustering within rows. However, there was a decrease in the percentage of clustered rows in replication 1 from the July 18 assessment date to July 25. The epidemic in the Machipongo field was devastating in 2007 and many of the rows in this trial had 100 percent disease incidence by the end of the season. Once disease incidence within a row reaches 100 percent the distribution is no longer considered significantly clustered according to Ordinary runs analysis, causing a decrease in the percentage of clustered rows in the last observation date of replication 1. Analyses of variance indicated significant differences in disease incidence and the percentage of rows showing a clustered distribution among the

assessment dates until the last three dates, July 11, July 18, and July 25 (Table 2). Results from the correlation analysis between disease incidence and the percentage of rows depicting a clustered distribution revealed a positive correlation (Table 1).

2008 Painter Trial. Similar results occurred in the 2008 trial as observed in previous trials (Fig. 4). The percentage of rows exhibiting a clustered distribution increased as the season progressed. The percentage of rows showing a significantly clustered distribution of diseased plants within rows was at least 95% by July 18. Most of the assessment dates had a significantly higher disease incidence than the previous assessment date (Table 2). In this trial there was no significant increase in the percentage of clustered rows after the third assessment date (July 3). There was a positive correlation between disease incidence and the percentage of clustered rows (Table 1).

Discussion:

Analyses of variance illustrated that as growing seasons progress, tomato bacterial wilt incidence steadily increases. These results indicate that disease pressure increases in steady increments rather than drastic disease peaks. Through the use of ordinary runs analysis it was determined that tomato bacterial wilt becomes more clustered within rows as the growing season progresses. Results from the correlation analysis for all four trials indicate a strong positive correlation between disease incidence and the percentage of rows showing a clustered distribution of disease.

The results from these trials indicate that a common factor (or factors) contributes to a clustered distribution within rows, rather than randomly. Analysis of the data across rows using ordinary runs did not show a high percentage (less than 20%) of clustering (data not presented), further indicating spread within rows is more important than across rows in the development of

bacterial wilt epidemics. The steady increase in disease incidence and rows with a clustered distribution within rows indicates secondary spread between adjacent plants is occurring. One of the major differences between within rows and across rows is the plant to plant proximity. Within row plant spacing is approximately 46 to 61 centimeters, whereas across the rows the plant spacing is approximately 1.8 meters. This greater distance between plants across rows may remove the root to root contact and prohibit secondary spread of this pathogen via plant roots.

Two possible factors that could explain the significant clustered distribution patterns within rows are in the irrigation water and/or secondary spread from bacterial exudate exiting roots. Research conducted in Brazil reported drip irrigation at varying frequencies significantly increased incidence of tomato bacterial wilt when compared to overhead irrigation, 42.5% versus 5.0% (6). The combination of drip tape irrigation, increasing the soil moisture around the root zones of plants, and the proximity of root zones from plant to plant provides an optimal environment to promote clustered distribution of diseased plants within rows. Further research should be conducted to examine if increased plant spacing, limiting the root to root contact of neighboring plants, or possible irrigation alternatives other than those currently employed could suppress this disease without hindering yields. Buddenhagen and Kelman (1) showed that periodic dry conditions have been shown to arrest disease development and slow the spread. Reducing the frequency of irrigation is one possible alternative to the irrigation regimes currently in use. The commercial growers on the ESV irrigate twice daily especially as tomato plants get larger and temperatures increase. Reduced frequency of irrigation would create dry periods within the beds that may reduce disease incidence or slow the spread of the pathogen within a field. However, when changing irrigation regimes it becomes imperative to ensure that plant health is maintained with new practices that could be considered. Reducing the frequency of

irrigation at the cost of plant health would not be an acceptable alternative to the current practices.

Literature Cited:

1. Buddenhagen, I.W. and A. Kelman. 1964. Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum*. Annual Review of Phytopathology 2:203-230.
2. Commercial Vegetable Production Recommendations, Virgnia. 2008. Virginia Cooperative Extension Publication 456-420. F124-137.
3. Gibbons, J. D. 1971. Nonparametric Methods for Quantitative Analysis. Holt, Rinehart, and Winston, New York. 463 pp.
4. Hayward, A.C. 1991. Biology and Epidemiology of Bacterial Wilt caused by *Pseudomonas solanacearum*. Annual Review of Phytopathology 29:65-87.
5. Kelman, A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum*. NC Agricultural Experiment Station. Technical Bulletin 99:194.
6. Madden, L. V., Louie, R., Abt, J. J., and Knoke, J. K. 1982. Evaluation of tests for randomness of infected plants. Phytopathology 72:195-198.
7. Marouelli, W.A., Lopes, C.A. and Silva, W.L.C. 2005. Incidence of bacterial wilt on processing tomato under drip and sprinkle irrigation. Horticultura Brasileira 23:320-333
8. McCarter, S.M. 1993. Bacterial Wilt. p. 28-29. in: Compendium of Tomato Diseases. J.B. Jones, J.P. Jones, R.E. Stall, and T.A. Zitter, eds. American Phytopathological Society, St. Paul, MN. 73 pp.
9. National Agricultural Statistics Service (NASS). 2009. United States Department of Agriculture. December 11, 2009. http://www.nass.usda.gov/Data_and_Statistics/index.asp.

Table 1. Correlation between disease incidence and percentage of rows showing a clustered distribution of tomato bacterial wilt for four trials conducted in Eastern Shore of Virginia commercial tomato fields from 2006 to 2008.

Field/year	$(R^2)^b$	R^a	Prob (r)
Painter 2006	0.48	0.69	0.0029
Painter 2007	0.85	0.92	0.0001
Machipongo 2007	0.60	0.77	0.0001
Painter 2008	0.83	0.91	0.0001

[b]R^2 = Coefficient of determination

[a]R = Correlation coefficient

Table 2. The mean percent disease incidence of tomato bacterial wilt and percentage of rows exhibiting a clustered distribution of disease for the four trials conducted on the Eastern Shore of Virginia in 2006, 2007 and 2008.

Date	% Disease Incidence	% Clustered rows
2006 Painter		
July 14	10.1 d [z]	50.00 b
July 26	26.2 c	82.50 a
August 3	40.3 b	90.00 a
August 14	64.6 a	96.25 a
2007 Painter		
June 14	0.9 g	3.75 e
June 21	2.1 fg	7.50 e
June 27	5.8 ef	32.50 d
July 3	8.7 e	47.50 c
July 11	16.3 d	71.25 b
July 19	26.9 c	87.50 a
July 26	35.8 b	96.25 a
August 1	42.1 a	98.75 a
2007 Machipongo		
June 21	15.2 e	53.75 c
June 28	32.1 de	69.50 b
July 2	39.3 cd	81.50 ab
July 11	55.3 c	95.25 a
July 18	70.6 ab	92.25 a
July 25	76.2 a	86.25 a
2008 Painter		
June 20	5.7 f	30.00 d
June 26	17.7 e	48.75 c
July 3	44.3 d	86.25 b
July 11	62.3 c	93.75 ab
July 18	70.3 bc	100.00 a
July 24	75.9 b	98.75 a
August 5	88.0 a	98.75 a

[z] Values followed by the same letter indicate no significant differences ($P = 0.05$) within each trial and within each column according to Fisher's LSD mean comparisons.

Figure 1. Percentage of rows exhibiting a clustered distribution of wilted plants within rows of a commercial tomato field over four assessment dates from the 2006 growing season in Painter, Va.

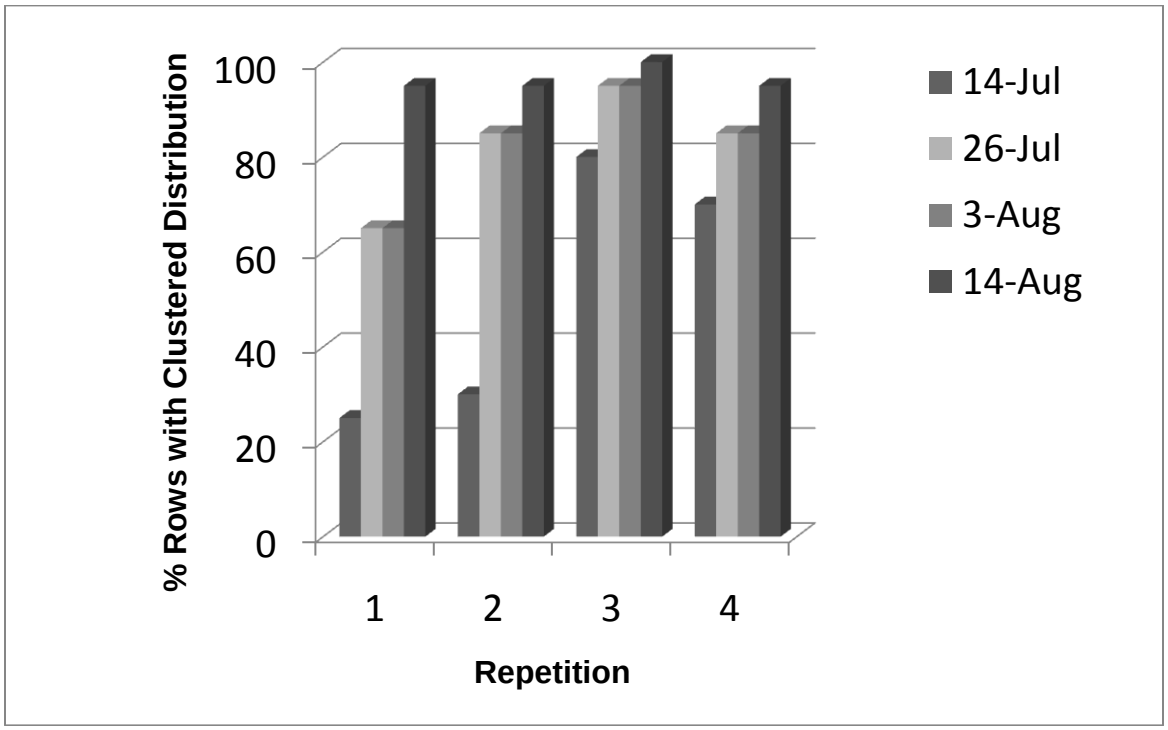


Figure 2. Percentage of rows exhibiting a clustered pattern of wilted plants within rows of a commercial tomato field over eight assessment dates from the 2007 growing season in Painter, Va.

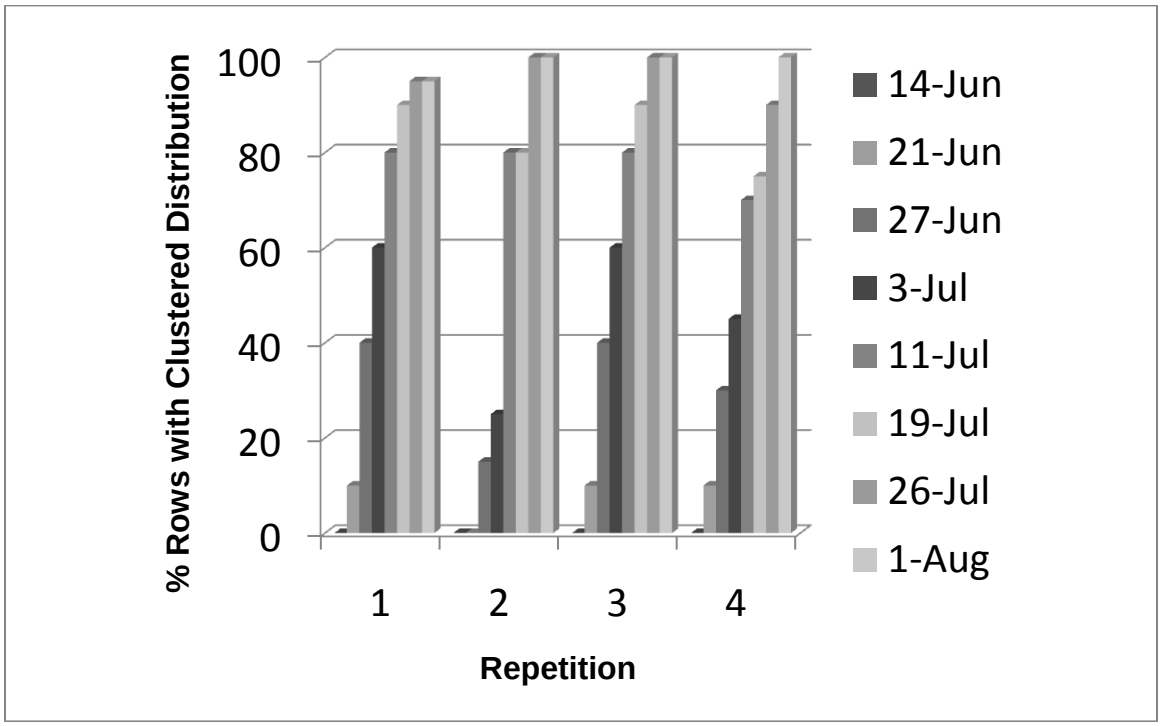


Figure 3. Percentage of rows exhibiting a clustered pattern of wilted plants within rows of a commercial tomato field over six assessment dates from the 2007 growing season in Machipongo, Va.

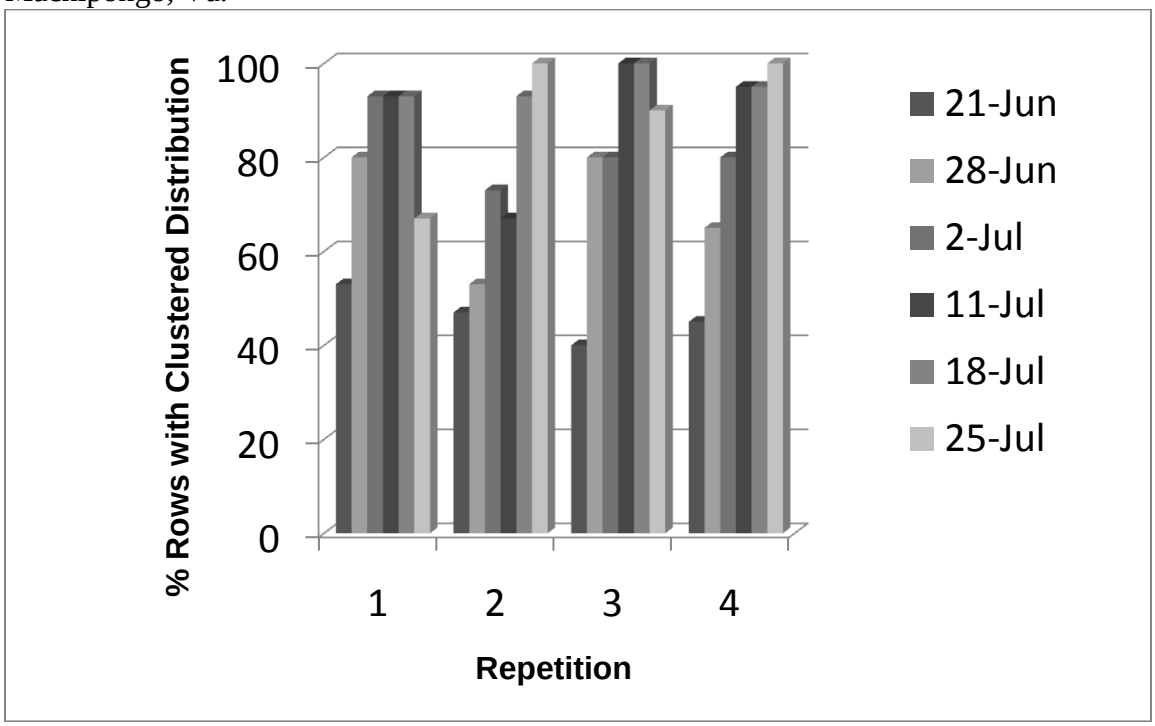
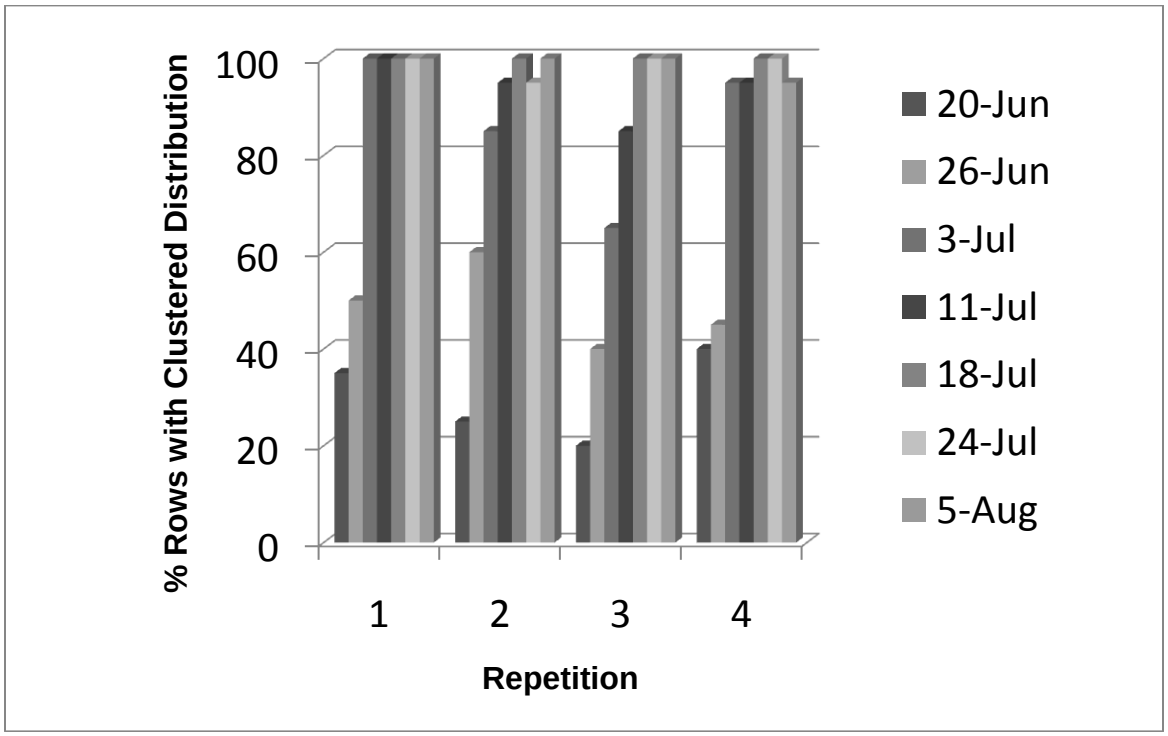


Figure 4. Percentage of rows exhibiting a clustered pattern of wilted plants within rows of a commercial tomato field over seven assessment dates from the 2008 growing season in Painter, Va.



Chapter 3:

Management of tomato bacterial wilt with grafted transplants, resistant cultivars, and breeding lines in conjunction with acibenzolar-*S*-methyl.

Introduction:

Bacterial wilt of tomato (*Solanum lycopersicum* L.), caused by *Ralstonia solanacearum* (Smith) Yabuuchi *et al*., is one of the most problematic diseases of tomato on the Eastern Shore of Virginia (ESV). Actual losses incurred by growers have not been recorded, but in certain fields, yield reductions of 50% have been observed. Crop rotation with non-susceptible crops has been the most effective management tactic in areas where the disease has been problematic. Tomato production on the ESV is unique; lack of available farm land does not allow growers to rotate crops and maintain profitability. Unless disease pressure can be suppressed, bacterial wilt threatens the sustainability of tomato production on the ESV. Successful management of this pathogen on the ESV will require an integrated approach with multiple control tactics.

Resistant varieties have been one of the most promising tools researchers have investigated to combat many tomato diseases and should be fully investigated for bacterial wilt management. To date, bacterial wilt-resistant cultivars that have been developed derive their resistance from one of three major sources. *Solanum pimpinellifolium* Jusl. is the source of resistance in the highly resistant Hawaii 7996, 7997, and 7998 breeding lines (5). A second source of resistance originates from a highly resistant landrace, S. *lycopersicum* var. *cerasiforme* (5). Venus and Saturn are two tomato cultivars derived from the third source of resistance, a cross between *S. pimpinellifolium* and Beltsville #3814 (5). The cultivars Venus, Saturn, and Neptune, introduced in the 1990's (8, 12), were the first commercially available cultivars to display some measure of resistance to bacterial wilt. Neptune originated from a heat tolerant line

(C1 11d x Campbell 28) crossed with a bacterial wilt resistant line (Hawaii 7997 x Florida 1C); thus, Neptune's resistance is from *S. pimpinellifolium* (5, 12). Growers have been slow to accept these bacterial wilt resistant tomato cultivars because they produce smaller fruit, appear less vigorous and often become too soft at maturation (9).

Tomato bacterial wilt can be suppressed through the use of a systemic acquired resistance inducer, acibenzolar-*S*-methyl (ASM) (Actigard 50WG, Syngenta Crop Protection, Greensboro, NC) (14). On the ESV, ASM is recommended for control of bacterial pathogens bacterial spot (*Xanthomonas campestris* pv. *vesicatoria* (Pammel) Dowson) and bacterial speck (*Pseudomonas syringae* pv. *tomato* Van Hall) (10). In greenhouse trials with low levels of inoculum, ASM reduced the incidence of bacterial wilt on the susceptible cultivar Solar Set (2). In the field, however, Pradhanang *et al.* (9) found ASM to be ineffective at reducing levels of bacterial wilt in susceptible cultivars, but among the tolerant cultivars Neptune and BHN-446, ASM significantly reduced disease incidence levels. However, they did not follow the current label instructions. The first application occurred two weeks after emergence as a foliar application, in the greenhouse, at a rate of 10 mL (10L/1000 plants) of ASM solution at 3µg/mL (9). A soil drench application was then applied with 5 mL (5L/ 1000plants) of ASM solution per plant at the same rate as the foliar application (9). They applied 0.03 g of product per 1000 plants in the foliar applications and 0.015g of product per 1000 plants in the soil drench applications. Following transplant foliar applications of ASM solution (30µg/mL) were made at 7 day intervals for 30 days, with each plant receiving 30 or 55 mL (358.64 L/ha or 657.51 L/ha) per application depending on the size of the plant (9). After the initial 30 days, plants were treated every 15 days with the same application rates for an additional 30 days (9). In field applications of ASM per plant were applied at a rate of 130.00 g/ha (30ml application) and 238.33 g/ha (55 ml

application) depending on the size of the plants. Araujo *et al.*, showed foliar and soil drench applications of Actigard 50WG enhanced resistance to the disease as well (3).

A new approach to soilborne disease management involves the use of grafting agronomically desirable scions onto resistant rootstocks. The technique of grafting scions of high yielding susceptible cultivars onto resistant rootstocks may even reduce the need for soil fumigants. Studies in Florida have demonstrated that grafting a susceptible tomato scion Bella Rosa onto the interspecific resistant rootstock Multifort significantly increased the yield of Bella Rosa compared to non-grafted and self-grafted plants (16). Zhao *et al.* (16) also showed that grafted transplants set into non-fumigated soil yielded similarly to non-grafted plants planted in fumigated (methyl bromide (MBr):chloropicrin (Pic), 50:50) soil. Grafting can be an effective control recommendation for growers with severe soilborne pathogens such as *R. solanacearum* and *Fusarium oxysporum* f.sp. *lycopersici* Sacc., Snyder and Hans. Rivard and Louws (11) conducted a trial to determine the effects of grafting in a field naturally infested with *R. solanacearum*. In trials conducted in 2005 and 2006, they found that the heirloom cultivar German Johnson showed no incidence of bacterial wilt when grafted onto resistant rootstocks of CRA 66 and Hawaii 7996 compared to the non-grafted treatment of German Johnson, which had 75to 79% incidence of bacterial wilt (11). They also reported similar findings with rootstocks resistant to fusarium wilt. Results of the trial indicated that fusarium wilt failed to develop when scions of German Johnson were grafted onto resistant rootstock Maxifort (11).

The use of resistant cultivars to suppress bacterial wilt has great potential. Although initial results have been promising, resistant cultivars are highly variable in resistance to this disease, especially in different geographical locations (5). Agronomic qualities of resistant cultivars are less desirable for growers. Grafted transplants offer a method in which fruit quality

can be maintained while offering disease resistance. The objective of this research was to investigate resistant breeding lines, grafting transplants and commercially available resistant cultivars to determine their ability to suppress bacterial wilt of tomato with and without ASM on the ESV.

Materials and Methods

Cultivar Trial in 2007. In the summer of 2007 a pilot trial was conducted examining eight different tomato cultivars (two susceptible and six resistant cultivars) in a commercial tomato field on the ESV. Transplants were produced in the greenhouse at Virginia Tech's Eastern Shore Agricultural Research and Extension Center in Painter. Greenhouse polystyrene trays of the inverted pyramid design (128 cells per tray with dimensions of 3.75x3.75x6.25 cm) (Speedling Inc., Sun City, FL), were filled with soil-less potting media (Promix BX, Premier Horticulture Inc., Rivière-du-Loup, Quebec) and seeded for each cultivar on June 7. Prior to transplanting, the plants were placed outside for a week so they could adjust to outdoor conditions.

Transplants were set in a commercial tomato field in Painter on July 18, 2007 and were placed into black polyethylene mulch that had been previously used for a spring crop of tomato. Bacterial wilt pressure in the previous crop over the trial area was 100%. Diseased plants from the previous crop were removed just prior to transplanting. The trial was arranged as a randomized complete block design with four replications per cultivar. Each plot consisted of a single row 4.6 meters long and contained 10 plants with an in-row spacing of 46 cm. Disease incidence ratings were recorded every 3 to 7 days beginning August 2. Due to the late planting date, yields were not recorded in this trial.

Preplant Field Preparations in 2008 and 2009. Field trials conducted in 2008 and 2009 were established in commercial tomato fields with histories of high bacterial wilt. Fields were fertilized to mirror commercial production practices. Methyl bromide:chloropicrin (67:33) at a rate of 112kg/ha in 2008 and 2009. Fumigants were shank injected with a single row combination bedder (Reddick Fumigants, Williamston, NC). Bedding and fumigation occurred on April 2 (spring) and June 23 (fall) for the 2008 trials and April 6 (spring) and July 7 (fall) for the 2009 trials. During fumigation, beds were covered with black, high density polyethylene mulch in the spring and metalized heat-strip polyethylene mulch (Pliant Corporation, Schaumburg Illinois) in the fall, both mulches were 1.25 mil. Drip tape for trickle irrigation was placed under the plastic at fumigation. Beds were 0.76 m wide, 15.24 cm high and had a between-row spacing of 1.8 m.

Transplant Production and Experimental Design for 2008 and 2009. Different cultivars, both susceptible and resistant to tomato bacterial wilt, were examined in these research trials (Table 1). Transplants were produced in the same manner as described in the 2007 pilot study. Greenhouse flats were seeded on March 31 for the Spring 2008 trial, June 2 for the Fall 2008 trial, April 1 for the Spring 2009 trial and June 19 for the Fall 2009 trial. For the grafted treatments, tomato seeds were planted into polystyrene flats of the inverted pyramid design on 5 March, 2008 and 24 March, 2009. Flat dimensions were 3.75x3.75x6.25 cm and were filled with soil-less media. Tomato seedlings were grafted at the two-leaf stage on 9 April, 2008 and 1 April, 2009. A tube graft was utilized and grafts were held together with 1.5 mm silicone grafting clips (Johnny's Selected Seeds, Winslow, ME). Rootstocks utilized were RST-04-105 (DP105) and RST-04-106 (DP106) (D. Palmer Seed Co., Yuma, AZ). Scions used were BHN-602 (BHN Seed, Immokalee, FL) and Sunguard (Seminis Seed, Saint Louis, MO). During the

first year of this experiment, it was observed that the growth of RST-04-105 was slower than that of RST-04-106 and BHN 602. It is crucial that the stem diameter of the rootstock and scion are similar, if not the graft junction may not heal correctly or the entire plant may die. The slow growth of DP105 prevented this during 2008 and the only available seedlings of the correct size were 'Sunguard'. In 2009, DP105 seeds were planted two weeks earlier than scion seed. A self graft of 'BHN 602' was also performed to ensure that the grafting process did not affect resistance.

Grafted transplants were healed in growth chambers kept at 27°C and 100% relative humidity. Plants were kept in total darkness for four days and then gradually exposed to light. After seven days in the growth chambers the grafted transplants were removed and placed in the greenhouse for an additional two weeks until transplanting. Transplanting dates for the Spring 2008 trial were May 7, July 25 for the Fall 2008 trial, May 28 for the Spring 2009 trial, and August 3 for the Fall 2009 trial. These trials were arranged in a split plot design with ASM treatment as the main plots and cultivar as the sub plots with four replications of each ASM treatment-cultivar combination. Individual plots were single rows at 9.14 m in length with plants spaced 46 cm apart in-row. Research trials were managed using Virginia Cooperative Extension recommendations for weed, disease, and insect control (4).

Acibenzolar-*S*-methyl Applications in 2008 and 2009. There were two main plot treatments in these trials: treatment with ASM or non-treated. Applications of ASM were made following label recommendations. Spray applications were made with a Spider Spray Trac (West Texas Lee Co., Idalou, Texas) at weekly intervals beginning one week after transplanting. The first two applications were sprayed at a total output of 467 l/ha (50 gal/A) at a rate of 23.34 g/ha (0.33 oz product/A) of Actigard 50WG. The spray nozzle arrangement included a drop

nozzle boom with a single spray tip on each side of the plants. Twin flat fan spray tips (Tee Jet Technologies, Wheaton, IL) were used in all Actigard 50WG applications. Applications for weeks three and four were applied at a total output of 655 l/ha (70 gal/A) at a rate of 35 g/ha (0.50 oz product/A). The spray nozzle arrangement was a drop nozzle boom with two spray tips on each side of the plants, one to cover the bottom half of the plants and a second to cover the top half of the plants. The last four applications were sprayed at a total output of 748 l/ha (80 gal/A) at a rate of 52.51 g/ha (0.75 oz product/A). In the final applications the same nozzle arrangement was used in weeks three and four with the addition of a fifth spray tip over top of the rows covering the very top of the plants. In the spring of 2008 the first application of ASM occurred on May 30, with seven more weekly applications ending on July 18. The first application of ASM in the fall trial of 2008 was on August 4 and seven subsequent weekly applications were made during the season with the final application occurring on September 22. In the spring trial of 2009, applications of ASM began on June 8 and the eight week spray program ended on July 28. The 2009 fall trial received the first application of ASM on August 12 and seven weekly sequential applications ending on October 3.

Data Collection and Analyses in 2008 and 2009. Incidence of bacterial wilt was recorded by plot at seven to ten day intervals once disease was present in the field. For each trial, mature green fruit were hand harvested, culled and sorted by size and marketability, according to the USDA grades and standards (1). In the Spring 2008 trial, plots were harvested on August 6 and 15, and October 20 and 29 for the Fall 2008 trial. The 2009 Spring trial was harvested on August 13 and 21 and the Fall 2009 trial was harvested once on October 21. Disease incidence and yield data were analyzed using ARM 7 (Gylling Data Management, Inc., Brookings, South Dakota). Disease incidence data was converted into area under the disease

progress curve (AUDPC) values (15). Analysis of variance was conducted on all data sets to determine whether significant ($p \leq 0.05$) main or sub plot treatment differences or main-sub plot interactions existed. Fisher's Least Significant Differences were used to separate significantly different treatments ($p = 0.05$).

Results

Cultivar Trial in 2007. Disease pressure for this trial was severe, with the susceptible cultivars Phoenix and Sebring showing more than 80 percent disease incidence at the end of the trial (September 4) (Fig. 1). The cultivars Neptune and FLA 7997 had over 25 percent disease incidence by the end of the trial. The Hawaii lines (HI 7997 and HI 7998) both had less than 20 percent disease incidence at the end of the trial. There were two breeding lines that exhibited less than 10 percent disease incidence by the end of the six weeks, CRA 66 and PI 126408. Less disease ($P = 0.05$) occurred in the breeding line PI 126408 compared to all of the other cultivars except HI 7998, HI 7997, and CRA 66 (Table 2). There was no yield collected for this trial.

Acibenzolar-*S*-methyl Effects in 2008 and 2009. Analyses of variance were performed to determine whether there were any significant interactions between the ASM treatments and cultivars in the 2008 and 2009 trials. No significant interactions were determined for all trials (p-values: $P = 0.4124$ for Spring 2008, $P = 0.3420$ for Fall 2008, $P = 0.7896$ for Spring 2009, and $P = 0.377$ for Fall 2009). Therefore treatments were analyzed across all ASM and cultivar treatments. No significant differences were observed in AUDPC values between ASM-treated and non-treated plots in either of the Spring trials (Table 3). Bacterial wilt incidence was very low in both Fall trials. No significant differences in yields were noted in any of the four trials based on ASM treatment.

Cultivar Effects in 2008 and 2009. Disease pressure was moderate in the Spring 2008 trial. Plants of the cultivar Sacramento were significantly more diseased than the other cultivars tested (Table 4). 'Red Line' also exhibited more disease than the resistant cultivars trialed in this study. Among the grafted treatments, self-grafted 'BHN 602' had significantly higher disease pressure compared to a 'BHN 602' or 'Sunguard' scion that was grafted onto resistant rootstocks. All other cultivars showed excellent bacterial wilt resistance. Fruit from 'CRA 66' and 'PI126408' were not considered marketable, thus, these two cultivars were not harvested. All three grafted treatments yielded poorly in this study due to high winds after transplanting that caused low survival among these treatments. The resistant cultivar BHN 669 yielded significantly better than all treatments except for 'FLA 8626'. Although total yield is important for determining a cultivar's agronomic characteristics, size of the fruit produced is important as well. 'BHN 669' produced the most total yield (by weight) compared to a majority of the other cultivars; however, 'FLA 8626' produced significantly more extra large tomatoes per plot than 'BHN 669' or any of the other cultivars in 2008 (Table 4).

For the Fall of 2008 'PI 126408' and 'CRA 66' were not harvested due to poor fruit marketability (Table 5). 'BHN 669' again exhibited the highest yield in this trial, significantly higher than all other cultivars except for 'FLA 7997'. The susceptible cultivar Red Line produced a yield significantly lower than 'BHN 669', but, was comparable to other cultivars and breeding lines. 'BHN 669' also produced significantly more pounds per plot of medium, large and extra large fruit than a majority of the other cultivars (Table 5). However, when considering pounds of extra large fruit, 'BHN 669' was not significantly different from 'FLA 8626', "FLA 8599', or the susceptible control of 'Redline'. Low levels of disease pressure were observed in the Fall 2008 trial.

Disease incidence was low in the Spring 2009 trial (Table 4). 'BHN602' grafted onto resistant rootstock DP105 resulted in AUDPC values that were significantly higher than all other cultivars. Yields were not assessed on 'PI 126408' and 'CRA 66' as with previous trials. 'BHN 669' produced the highest numerical yield; however, it was not statistically higher than 'Red Line', 'FLA 8599', 'FLA 8626', 'BHN 682', and two grafted treatments (BHN 602 on DP105 and BHN 602 on DP106). The greatest amount of extra large fruit was produced by 'FLA 8626', which was significantly more than all of the other cultivars in the study except for 'FLA 8599' (Table 4).

In the fall trial of 2009 there was no significant disease pressure. The trial was only harvested once, October 21, and produced low yields due to poor growing conditions (Table 5). In the fall trial of 2009 'FLA 8599' produced significantly more yield than a majority of the other cultivars tested. However, yields of 'FLA 8599' were not significantly higher than 'Red Line' or 'FLA 8626'. 'FLA 8599' yielded significantly more pounds per plot of extra large fruit compared to all of the other cultivars except 'FLA 8626' (Table 5).

Discussion:

Tomato bacterial wilt is particularly devastating to ESV tomatoes in the spring season. Fall tomato production on the ESV typically does not have the disease intensity from bacterial wilt compared to the spring season. Several factors may contribute to the limited disease pressure in the fall crop compared to the spring. First and foremost the soil that is used in fall production is exposed to more extreme temperature and moisture trends than the soil used in spring production. During the spring season the soil that will be used for fall production is left uncovered and exposed to high heat and limited moisture which dries out the soil. Dry soil decreases the pathogens ability to survive (6). The prolonged exposure to high temperatures

without a continuous source of moisture (irrigation) can cause the soil populations of *R. solancaearum* to decline in the upper layers of the soil. This reduction in pathogen population in the top portion of the soil could explain the limited activity *R. solanacearum* has on tomato production in the fall. The soil that is used for spring production is covered with polyethylene mulch, prior to the high temperatures in the summer. The mulch traps in moisture keeping the soil underneath moist while it heats up. The trapped moisture and increased heat provides the environment *R. solanacearum* requires for survival and reproduction (6). Thus, soil conditions in the tomato beds may be much more conducive for pathogen survival, infection, and spread during the spring growing seasons compared to those in the fall. Planting date is a factor that should be examined more in future tomato bacterial wilt research trials on the ESV. Unfortunately, ESV growers plant on a continuum from spring to fall to ensure that they have tomato fruit across the entire growing season. Therefore planting date cannot be used solely as a management tactic, but should be included as a component in an integrated pest management program.

Research by Pradhanang *et al.*, showed greenhouse and field applications of ASM enhanced the resistance of moderately resistant cultivars to tomato bacterial wilt (9). Researchers included a foliar application and a soil drench application in the greenhouse prior to transplanting (9). The results from Pradhanang *et al.* were supported by the findings from Ji. P *et al*. (7, 9). However, from our research trials conducted on the ESV, labeled applications of ASM did not enhance resistance to bacterial wilt. The results from Pradhanang *et al.* and Ji. P *et al*. compared with the findings in these studies indicate that for ASM to enhance resistance to bacterial wilt, cultivars need to be exposed prior to transplanting (7, 9). Other research further supports this pre-exposure to ASM for the resistance response to be effective in plants. Soylu *et*

al. (13) found that tomato seedlings pretreated with ASM prior to exposure of bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis *et al.*) had up to 75 percent reduction in disease severity compared to those plants that were not treated with ASM. Although not included in our research, pre-exposure applications of ASM should be further investigated. Extensive trials looking at the potential harmful effects of ASM on tomato seedlings as well as enhanced resistance should be conducted to determine the threshold of maximum control and least amount of phytoxicity.

Performance of grafted transplants was variable in our trials. Weather conditions in 2008 had a detrimental effect on grafted plant yield, despite these treatments (DP105 and DP106) showing effective disease resistance. Late pruning of the resistant rootstocks also contributed to low yields. Resistant rootstocks that were not pruned properly in the trial out-grew the scion and utilized the majority of nutrients, sunlight, and water, contributing to the low yields observed in this trial. Although replanting occurred, these treatments never sufficiently recovered. Care must be taken to ensure proper timing of seeding for compatible rootstock and scions as well as pruning after transplanting. In the Spring 2009 trial DP105 had significantly more disease than the other cultivars examined. The reported disease incidence indicates that DP105's resistance was not maintained throughout the growing season. This loss of resistance is unexpected after the results from the 2008 spring trial. Possible explanations of this lack of resistance could be a rootstock mix up from the seed company. Another possible explanation is the pathogen was present in overwhelming numbers in some of the plots where this rootstock was planted. The discrepancies between the 2008 and 2009 trial do not indicate a failure in the resistance of the rootstock. The rootstock did not maintain its resistance in the 2009 trial with overall lower disease pressure than the 2008 trial. The most likely answer to this discrepancy is a mix up in

rootstocks from the seed source. Although promising, further research needs to focus on grafting commercial tomato transplants that can tolerate field conditions and that are effective against *R. solanacearum*.

Results from these studies indicate that resistant cultivars are available and cultivars being developed can maintain bacterial wilt resistance throughout a growing season. Although the better cultivars in the trials performed well in maintaining resistance such as 'BHN 669', 'FLA 7997', 'FLA 8599' and 'FLA 8626' these cultivars need to improve in fruit yield and quality. 'BHN 669' is the most promising available cultivar for ESV tomato growers to incorporate into production practices at this time. Limited production of 'BHN 669' occurred on the ESV in 2009 with moderate acceptance by growers. Common problems with these cultivars included smaller fruit, soft fruit, fruit with large shoulders giving them an odd shape, and overall lower yields. 'BHN 669' produced the highest marketable yield throughout most of the trials; however, the fruit size was not as large as some of the other cultivars examined in this study. Although the overall yields were lower, 'FLA 8626' and 'FLA 8599' produced significantly larger fruit in most of the trials. Agronomic characteristics such as fruit size are paramount to commercial tomato growers. While these breeding lines and cultivars possess promising disease resistance, these problems need to be corrected before growers will utilize these cultivars.

Literature Cited:

1. Agricultural Marketing Service. 2009. United States Department of Agriculture. December 14, 2009. http://www.ams.usda.gov/AMSv1.0/ams.fetchTemplateData.do?template=TemplateN&page=FreshMarketVegetableStandards
2. Anith, K.N., M.T. Momol, J.W. Kloepper, J.J. Marois, S.M. Olson, and J.B. Jones. 2004. Efficacy of plant growth-promoting rhizobacteria, acibenzolar-*S*-methyl, and soil amendment for integrated management of bacterial wilt on tomato. Plant Disease 88:669-673.
3. Araujo, J.S. de P., Goncalves, K.S., Ribeiro, R. de L.D., Polidoro, J.C. and Rodrigues, R. 2005. Resistance to tomato bacterial wilt induced by acibenzolar–S–methyl. Acta Horticulturae 695: 429-34.
4. Commercial Vegetable Production Recommendations, Virgnia. 2008. Virginia Cooperative Extension Publication 456-420. F124-137.
5. Hanson, P. M., Licardo, O., Hanudin, Wang, J. F., and Chen, J.-T. 1998. Diallel analysis of bacterial wilt resistance in tomato derived from different sources. Plant Disease 82:74-78.
6. Hayward, A.C. 1991. Biology and Epidemiology of Bacterial Wilt caused by *Pseudomonas solanacearum*. Annual Review of Phytopathology 29: 65-87.
7. Ji, P, Momol, M.T., Olson, S.M., Hong, J., Pradhanang, P., Anith, K.N. and Jones, J.B. 2005. New tactics for bacterial wilt management on tomatoes in the Southern U.S. Acta Horticulturae 695: 153-59.
8. McCarter, S.M. 1993. Bacterial Wilt. p. 28-29. in: Compendium of Tomato Diseases.

J.B. Jones, J.P. Jones, R.E. Stall, and T.A. Zitter, eds. American Phytopathological Society, St. Paul, MN. 73 pp.

9. Pradhanang, P.M., P. Ji, M.T. Momol, S.M. Olson, J.L. Mayfield, and J.B. Jones. 2005. Application of acibenzolar-*S*-methyl enhances host resistance in tomato against *Ralstonia solanacearum*. Plant Disease 89:989-993.

10. Rideout, S. L., Waldenmaier, C. F., Wimer, A. F., and McDuffie, J. B. 2007. Evaluation of fungicides and bactericides applied with and without Actigard for control of bacterial spot in staked tomato, 2006. Plant Disease Management Reports. No. 1:V152.

11. Rivard, C. L., and Louws, F. J. 2008. Grafting to Manage Soilborne Diseases in Heirloom Tomato Production. Hortscience 43(7):2104–2111.

12. Scott, J. W., Jones, J. B., Somodi, G. C., Chellemi, D. O., and Olson, S. M. 1995. 'Neptune', a heat-tolerant, bacterial wilt-tolerant tomato. HortScience 30:641-642.

13. Soylu, E. M., Soylu, S., and Baysal, O. 2003. Induction of disease resistance and antioxidant enzymes by acibenzolar-S-methyl against bacterial canker (Clavibacter michiganensis subsp michiganensis) in tomato. Journal of Plant Pathology 85:175-171.

14. Tally, A., M. Oostendorp, K. Lawton, T. Staub, B. Bassi. 1999. Commercial development of elicitors of induced resistance to pathogens. p. 357-370 in: Induced Plant Defense Against Pathogens and Herbivores. Biochemistry, Ecology and Agriculture. A.A. Agrawal, S. Tuzun, and E. Bent, eds. American Phytopathological Society, St. Paul, MN.

15. Vanderplank, J. E., 1963. Plant Diseases: Epidemics and Control. Academic, New York,

344 p.

16. Zhao, X., Simonne, E. H., Hochmuth, R. C. Grafting as an Alternative to Methyl Bromide in Field Tomato Production. Oral Session Abstracts. 106th Annual International Conference of the American Society for Horticultural Science, 2009.

Table 1. List of susceptible and resistant cultivars and their sources included in the research trials examining resistant cultivars on the Eastern Shore of Virginia, in 2007, 2008, and 2009.

Cultivars	Source
Susceptible	
BHN 602	BHN Seed, Immokalee, FL
Redline	Syngenta Seeds, Golden Valley, MN
Phoenix	Seminis Vegetable Seeds Inc., Saint Louis, MO
Sebring	Syngenta Seeds, Golden Valley, MN
Sunguard	Seminis Vegetable Seeds Inc., Saint Louis, MO
Resistant	
CRA 66	National Seed Storage Lab, Fort Collins, CO
PI 126408	National Seed Storage Lab, Fort Collins, CO
HI 7997	National Seed Storage Lab, Fort Collins, CO
HI 7998	National Seed Storage Lab, Fort Collins, CO
FLA 7997	University of Florida, Gainesville, FL
FLA 8109B	University of Florida, Gainesville, FL
FLA 8599	University of Florida, Gainesville, FL
FLA 8626	University of Florida, Gainesville, FL
BHN 669	BHN Seed, Immokalee, FL
BHN 682	BHN Seed, Immokalee, FL
Sacramento	D. Palmer Seed Company, Yuma, Az
Neptune	University of Florida, Gainesville, FL
Resistant Rootstocks	
RST-04-105	D. Palmer Seed Company, Yuma, Az
RST-04-106	D. Palmer Seed Company, Yuma, Az

Table 2. Disease incidence area under disease progress curve (AUDPC) values of the pilot study conducted in 2007 examining the resistance of several cultivars and breeding lines to tomato bacterial wilt in Painter, Va.

Cultivar	Description[z]	Disease Incidence (AUDPC)
Phoenix	S	111.00 a[y]
Sebring	S	121.25 a
PI 126408	R	3.13 d
CRA 66	R	10.63 cd
HI 7997	R	15.00 cd
HI 7998	R	20.38 bcd
Neptune	R	46.75 bc
FLA 7997	R	59.13 b

[z] Indicates description of cultivar, S = susceptible R = resistant cultivar

[y] Values followed by the same letter indicate no significant differences according to Fisher's Least Significant Differences ($P = 0.05$)

Table 3. Bacterial wilt incidence area under disease progress curve (AUDPC) and yield of treatments that either received acibenzolar-*S*-methyl (ASM) treatments or were not treated (NTC) across tomato cultivars for four trials conducted on the Eastern Shore of Virginia.

Trial	Treatment[z]	Disease Incidence (AUDPC)	Yield 25 lb Boxes/A
Spring 2008	ASM	46.5 a[y]	627 a
	NTC	36.2 a	742 a
Fall 2008	ASM	NDP	1162 a
	NTC	NDP	1367 a
Spring 2009	ASM	2.20 a	1111 a
	NTC	9.42 a	1158 a
Fall 2009	ASM	NDP	148 a
	NTC	NDP	167 a

[z] NTC = non-treated control; ASM = treatments of Actigard 50WG

[y] Values followed by the same letter indicate no significant differences within each trial and within each column according to Fisher's Least Significant Differences ($P = 0.05$) NDP = no disease present

Table 4. Bacterial wilt disease incidence area under disease progress curve (AUDPC) and yield for the individual tomato cultivars included in the trials conducted in Painter, Va in the spring of 2008 and 2009.

Cultivar	Description[z]	Disease Incidence	Medium Fruit Wt (lbs/plot)	Large Fruit Wt (lbs/plot)	Extra Large Fruit Wt (lbs/plot)	Total Yield 25 lb Boxes/A
Spring 2008						
Red Line	S	76. 6 c[y]	4.37 c	20.22 c	74.14 b	956 bc
CRA 66	R	0 d	0 d	0 e	0 e	0 f
FLA 7997	R	9.5 d	24.21 a	34.33 b	28.20 cd	840 cd
FLA 8109B	R	11.6 d	0.52 d	7.36 de	46.36 c	525 de
FLA 8599	R	0.7 d	2.07 d	15.05 cd	80.45 b	944 bc
FLA 8626	R	2.1 d	0.74 d	10.84 cde	116.73 a	1242 ab
PI 126408	R	1.6 d	0 d	0 e	0 e	0 f
BHN 682	R	7.4 d	17.49 ab	42.40 ab	47.20 c	1037 bc
BHN 669	R	12.4 d	18.51 ab	48.93 a	85.58 b	1481 a
Sacramento	R	145.8 a	11.41 bc	17.33 cd	12.16 de	396 e
Sunguard on 105	G	8.8 d	4.81 cd	14.19 cd	35.28 cd	525 de
BHN 602 on 106	G	12.3 d	5.11 cd	13.71 cd	38.87 c	558 de
BHN 602 on BHN 602	G	113.4 b	3.89 d	9.07 cde	27.48 cd	392 e
Spring 2009						
Red Line	S	3.75 b	17.46 c	44.8 bc	68.28 b	1554 ab
CRA 66	R	0.00 b	0 f	0 g	0 g	0 e
FLA 7997	R	0.75 b	31.08 a	42.44 bc	31.53 de	1235 bcd
FLA 8109B	R	0.00 b	11.36 d	35.22 cd	46.46 cd	1162 bcd
FLA 8599	R	0.00 b	9.08 de	27.97 de	74.43 ab	1459 abc
FLA 8626	R	1.50 b	6.21 d	20.10 ef	85.35 a	1300 abcd
PI126408	R	0.00 b	0 f	0 g	0 g	0 e
BHN 682	R	0.75 b	25.14 b	54.14 ab	48.76 c	1552 ab
BHN 669	R	0.00 b	25.77 ab	58.11 a	67.60 b	1795 a
Sacramento	R	7.75 b	19.38 c	28.04 de	24.75 ef	852 d
BHN 602 on 105	G	58.19 a	14.99 cd	41.90 c	60.59 bc	1431 abc
BHN 602 on 106	G	2.88 b	8.89 de	22.87 ef	31.04 def	1418 abc
BHN 602 on BHN 602	G	0.00 b	4.18 ef	10.84 fg	16.94 f	990 cd

[z] Indicates description of cultivar, S = susceptible, R = resistant cultivar, G = grafted treatment

[y] Values followed by the same letter indicate no significant differences within each trial and within each column according to Fisher's Least Significant Differences ($P = 0.05$)

Table 5. Yield for the individual tomato cultivars included in the trials conducted in Painter, Va in the fall of 2008 and 2009.

Cultivar	Description[z]	Medium Fruit Wt (lbs/plot)	Large Fruit Wt (lbs/plot)	Extra Large Fruit Wt (lbs/plot)	Total Yield 25 lb Boxes/A
Fall 2008					
Red Line	S	8.30 abc[y]	29.63 b	63.36 a	1303 bc
CRA 66	R	0 e	0 e	0 c	0 e
FLA 7997	R	8.04 abcd	25.95 b	30.86 b	1530 ab
FLA 8109B	R	5.20 bcd	13.81 cd	22.52 b	912 d
FLA 8599	R	3.51 de	13.59 cd	55.53 a	1453 b
FLA 8626	R	3.98 cde	10.87 d	73.71 a	1191 c
PI 126408	R	0 e	0 e	0 c	0 e
BHN 669	R	11.93 a	42.03 a	74.35 a	1754 a
Sacramento	R	9.02 ab	22.77 bc	23.87 b	727 d
Fall 2009					
Red Line	S	1.93 ab	6.77 a	4.72 bc	260 ab
CRA 66	R	0 d	0 c	0 e	0 d
FLA 7997	R	0.97 d	2.24 b	1.11 de	84 c
FLA 8109B	R	1.31 cd	2.24 b	0.78 de	84 c
FLA 8599	R	2.69 abc	6.08 a	7.66 a	318 a
FLA 8626	R	1.48 bc	5.43 a	6.92 ab	268 ab
PI 126408	R	0 d	0 c	0 e	0 d
BHN 669	R	3.16 a	6.20 a	3.11 cd	241 b
BHN 682	R	2.30 abc	5.57 a	1.51 de	182 bc
Sacramento	R	2.74 ab	3.28 b	1.19 de	140 c

[z] Indicates description of cultivar, S = susceptible, R = resistant cultivar

[y] Values followed by the same letter indicate no significant differences within each trial and within each column according to Fisher's Least Significant Differences ($P = 0.05$)

Figure 1. Incidence of tomato bacterial wilt in eight tomato cultivars in a commercial tomato field in Painter, Va for the 2007 pilot study. Letters following the name of the cultivars indicate resistance, S indicates susceptible cultivars and R indicates resistant cultivars.