Biorational approaches for management of bacterial wilt and bacterial spot on tomato

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Coordinators:

Jeffrey Jones
Professor
University of Florida
Plant Pathology Department
Gainesville, FL 3261
352-273-4673
E-mail: jbjones@ufl.edu

Summary

We have identified several alternative approaches for improved control of bacterial spot and bacterial wilt. These approaches have either relied on finding alternative control strategies that rely on totally on environmentally-friendly control strategies in the case of bacterial wilt or in the case of bacterial spot of tomato, we have identified strategies for applying combinations of chemicals and/or biological control agents. For bacterial wilt, we determined that the application of thymol and acibenzolar-S-methyl when was used together in field conditions to control bacterial wilt on moderately resistant tomato cultivars will not have a negative affect on the tomato production. The combination of both products numerically increased the fruit yield and decreased the disease incidence for the susceptible cultivar. For bacterial spot, we looked at reduced rates of actigard. In the reduced rate experiment 1/10 X rate of Actigard effectively controlled bacterial spot on the tolerant line (8314) compared to the standard copper bactericide treatment early in the experiment; however, as the experiment continued, Actigard provided no improved disease control. The tolerant genotype had significantly less disease than the susceptible genotype (Bella Rosa) at the end of the experiment. In general by the end of the experiment none of the treatments in the tolerant variety were significantly different. Yield for all treatments were not significantly different. In a separate reduced risk experiment twice weekly applications of bacteriophage provided significantly better disease control than the standard copper-mancozeb treatment. Growers may wish to consider using bacteriophages as a treatment for bacterial spot of tomato as an alternative to the standard copper-mancozeb treatment. We also provide evidence for bacterial spot disease control with a phosphorous acid salt. Clearly more work is necessary for finding improved strategies for disease control of bacterial spot and bacterial wilt; however, we do provide information for improved control strategies that in some cases

growers are beginning to accept.

Introduction
Statement of Problem, Rationale, and Significance

The purpose of this project is to develop environmentally-compatible approaches for management of bacterial wilt and bacterial spot, two important diseases on tomatoes. Tomato is the most significant vegetable crop in the southern U.S. totaling more than one billion U.S. dollars in farm gate value with Florida accounting for close to 65% of the fresh market production (Annual report of Florida Tomato Committee, 2005). However, tomato yield and quality are threatened by bacterial wilt and spot for which effective control measures are limited. Bacterial wilt, caused by Ralstonia solanacearum, is probably the most important bacterial disease of plants in tropical, subtropical and warm temperate zones of the world. Bacterial spot, incited by Xanthomonas campestris pv. vesicatoria, is a major problem in Florida as well as tropical, subtropical and temperate environments where high temperatures and moisture conditions prevail during the growing season. Bauske et al. (1998) identified bacterial spot of tomato as a major production problem in the southeastern United States. The Florida Tomato Committee and North Florida Tomato Growers have prioritized bacterial wilt and bacterial spot, respectively, as high priority research areas. Bacterial wilt is a systemic disease in which infected plants do not respond to treatments such as copper/mancozeb. It is also difficult to eliminate from fields since the pathogen persists in a wide range of crop and weed hosts (Kelman, 1953). Race 1 is associated with bacterial wilt in tomato, potato and other solanaceous hosts in the U.S. In addition to the loss of plants and yield, another serious impact of bacterial wilt in the southeastern U.S. is the abandonment of previously productive farms due to serious outbreaks of the disease. Use of conventional general-purpose soil fumigants such as methyl bromide not only has been ineffective in controlling bacterial wilt (Chellemi et al., 1997), but also poses a threat to beneficial microorganisms in the soil-root micro-ecosystems. Bacterial spot causes frequent and severe yield and quality losses in fresh-market and processing tomato production (Pernezny et al., 1996). In Florida, the disease is endemic (Jones et al., 1986) and is caused primarily by X. campestris pv. vesicatoria tomato races 1 and 3 (Jones et al. 1995; Jones et al., 1998). Antibiotics such as streptomycin have been ineffective for many years as a result of the development of resistant strains (Thayer and Stall, 1961). During the fall seasons growers usually apply copper plus mancozeb at least twice weekly in an attempt to control this disease. However, the disease is not controlled when environmental conditions are optimal for disease development (Jones et al., 1991; Marco and Stall, 1983). Furthermore, control is hampered in part by the presence of copper-tolerant strains and the endemic nature of the pathogen in some locations (Ritchie and Dittapongpitch, 1991; Stall et al, 1986). Development of biorational alternatives is hence desirable for more effective control of bacterial spot and for reduction of copper accumulation in the soil. In this project, we intend to develop effective approaches integrating several new and promising findings to reduce incidence of bacterial wilt in the field. Environmentally friendly tools such as plant essential oils or plant extracts will be used as pre-transplanting treatments to reduce soil-borne pathogen inoculum. Systemic acquired resistance (SAR) inducers such as Actigard will be used to trigger natural host resistance prior to and post tomato transplanting. The bacterial wilt pathogen is difficult to manage since it persists in soils over long periods or survives in soils for extended periods in association with infested plant debris (Granada and Sequeira, 1983), which provide inoculum sources between growing seasons. Hence, preplant soil treatment using adequate biofumigants or other reduced risk compounds to eliminate or reduce soil populations of the pathogen will be an appropriate strategy in management of this disease. As part of our previous research, we evaluated the use of plant essential oils that were shown to be effective antimicrobials against soil-borne fungi and nematodes to manage bacterial wilt in tomatoes. Among the essential oils, thymol had significant activity against R. solanacearum in in vitro studies (Momol et al., 1999). In greenhouse experiments, thymol efficiently eliminated R. solanacearum soil populations and effectively controlled bacterial wilt (Pradhanang et al., 2003). In recent field tests, thymol was shown to be an effective biofumigant to reduce bacterial wilt incidence significantly on susceptible cultivars and resulted in higher yields (Ji et al., 2005). Thymol has great promise as an effective component in integrated management of bacterial wilt of tomato. However, studies are needed to establish and optimize field application methodology. Efficacy of thymol in control of bacterial wilt on commercially available tolerant or resistant tomato cultivars deserves evaluation. Furthermore, the effect of thymol in R. solanacearum infested soils representative of heavy natural infestation needs to be investigated. Other reduced risk compounds also deserve to be evaluated for efficacy and compared with thymol as complementary methods for soil treatment. Some commercial products containing primarily plant extracts provided effective protection against bacterial wilt when used as soil treatment. In a greenhouse study, tomato plants grown in Yucca schidigera extract-treated soils had significantly lower bacterial wilt incidence compared with the untreated control (Momol et al., unpublished). A series of in vitro studies indicated that several other natural products, including carvacrol, geraniol, linalool and the fungal biological control agent Muscodor that produces volatile compounds, completely inhibited growth of R. solanacearum on agar medium. Further greenhouse and field studies are necessary to evaluate efficacy of these products as biofumigants for reducing soil populations of R. solanacearum and bacterial wilt on tomato. In addition, soil treatment with other biorational products such as Serenade showed significant efficacy against bacterial wilt under greenhouse conditions (Momol et al., unpublished). Further evaluation of these natural or biological products under greenhouse and field conditions has the potential to develop new environmentally-safe approaches for effective control of bacterial wilt. Host resistance is an efficient and effective component in integrated management of bacterial wilt. Varieties and genotypes with moderate resistance to bacterial wilt have been developed in the University of Florida breeding program, such as 'Neptune' (Scott et al. 1995) and hybrids from 'Neptune' like FL7514. In greenhouse and field studies, the plant activator Actigard (Syngenta Crop Protection, NC) significantly enhanced resistance of tolerant cultivars, including Neptune and FL7514, against bacterial wilt and resulted in increased tomato yield (Pradhanang et al., 2005). While integrated use of Actigard and tolerant tomato cultivars showed great potential in bacterial wilt management, only limited research has been done on commercially available cultivars to optimize Actigard application for economic benefit. On susceptible cultivars, studies indicated that Actigard was more effective in reducing bacterial wilt incidence at low soil populations of R. solanacearum (Anith et al., 2004). So far, it is unknown if Actigard provides effective control of bacterial wilt on susceptible cultivars under field conditions when relatively lower R. solanacearum inoculum exists. Hence, the efficacy of Actigard in controlling bacterial wilt on susceptible cultivars needs to be evaluated under field conditions with R. solanacearum inoculum levels similar to that typically encountered in naturally infested fields. In addition, integration of Actigard with other promising tactics such as thymol, yucca extracts or other biorational products that reduce soil populations of the pathogen has the potential for further improving disease suppression. Several alternative disease management strategies have efficacy against bacterial spot. Among these, bacteriophages (Flaherty et al., 2000), bacterial antagonists (Byrne et al., 2005) and Actigard (Louws et al. 2001) are most promising. Another alternative method is the use of plant growth promoting rhizobacteria (PGPRs) to activate plant systemic resistance responses, which has been useful in reducing bacterial speck of tomato and other bacterial diseases. Experiments in the bacterial spot-tomato system have not shown much promise (Jones et al., unpublished; Obradovic et al., 2005). However, we have observed enhanced activation of SAR towards X. campestris pv. vesicatoria T1 and T3 strains in two genotypes which were selected for resistance to T2 (Jones et al., unpublished). Bacteriophages have significant potential as biocontrol agents for controlling bacterial diseases. Phages were used to control Stewart's wilt disease in corn caused by Pantoea stewartii (Thomas, 1935). Civerolo and Kiel (1969) reduced bacterial spot of peach seedlings, caused by Xanthomonas pruni, by phage applications. However, phages were abandoned due to the emergence of bacterial mutants resistant to the phages. An approach that uses a mixture of bacteriophages was developed to overcome problems experienced in the past (Jackson, 1989). We tested mixtures of bacteriophages for control of bacterial spot on overhead irrigated tomato transplants and observed that irrigation water containing bacteriophages specific to the bacterial spot pathogen reduced disease incidence compared to a copper bactericide (Somodi et al., 1997). In further field studies, bacterial spot severity was significantly reduced on phage-treated plants, especially formulated bacteriophages, compared to those receiving copper-mancozeb or the control (Flaherty et al., 2000; Balogh et al., 2003) while yield was also significantly improved. Studies integrating bacteriophages and other biorational measures have the potential to develop effective approaches as alternatives to copper bactericides for bacterial spot management. In field studies where Actigard was applied, bacterial spot disease severity was significantly reduced on tomatoes without significant yield responses (Louws et al. 2001). In another study conducted in Florida, Actigard effectively reduced disease without significantly improving yields (Obradovic et al., 2004). In greenhouse studies applications of Actigard stimulated defense responses as manifested by development of necrotic lesions on leaves which in further tests was determined to result from a hypersensitive response (Obradovic et al., 2005). Given that Actigard applications activate strong defense responses, further research is needed on application rates and timing to reduce negative effects on yield responses. Host resistance to pathogens is a critical component of integrated disease management systems. Resistance to the bacterial spot pathogen is quite extensive (Jones et al., 1995; Scott and Jones, 1986; Scott et al., 1995; Scott et al., 1997). Furthermore, a transgenic tomato genotype has been developed that contains the Bs2 resistance gene, which confers resistance against all known Xanthomonas strains that attack tomato (Tai et al., 1999). Further complicating the use of plant-derived resistance to control bacterial spot is the fact that X. campestris pv. vesicatoria composes a very heterogeneous group of strains (Bouzar et al., 1994). Romero and Ritchie (2004) observed that Actigard effectively delayed race changes and may increase the durability of genotype-specific resistance. Therefore, deployment of varieties with moderate to high levels of resistance to bacterial diseases coupled with the use of SAR inducers may increase durability of these resistances. In this proposed project we plan to expand the use of these newly available tools to include the bacterial wilt pathogen and two races of the bacterial spot pathogen. The main purpose of this project is to evaluate new and/or alternative bacterial disease management approaches and to integrate the most promising ones into comprehensive, environmentally and economically viable strategies to manage these two major tomato bacterial diseases. Management of bacterial wilt of tomato was not addressed in previous SARE-funded projects. Control of bacterial spot of tomato by compost, microbial biocontrol agents and Actigard was studied by researchers in North Central region (projects: LNC96-099, LNC98-141). However, bacteriophages and integration of resistant tomato lines with PGPRs have not been investigated. Through the new strategies developed in this proposed project, a more sustainable tomato production will be established, which will be readily accepted by tomato growers (small and mid size farm with owner operated). Due to the destructive nature of these diseases, growers usually apply copper bactericides for bacterial spot and conventional fumigants for bacterial wilt. Some of the proposed tactics in this project will help to reduce such standard but wasteful use of pesticides. Potential users of this research are farmers, IPM providers and extension personnel in the southeastern US. This mission-oriented project supports the long-range goal of improving the sustainability of U.S. agriculture by reducing high risk chemical use through environmentally reduced-risk tactics. Sustainable Agriculture Relevance The proposed project aims at development of effective and practical tools for management of bacterial wilt and bacterial spot on tomatoes, an important food crop in the U.S. Both bacterial wilt and bacterial spot cause significant yield loss and practical measures are lacking for effective control of the two diseases. Development and implementation of the innovative tactics proposed in this project to reduce losses to the diseases will be beneficial to maintain safe food supply. Bacterial wilt is a devastating disease on tomato and many other food crops, and, the pathogen persists in soils for extended periods of time once the soil is infested. For nearly 100 years, tomato growers in the southern U.S. have abandoned production fields due to infestation of the pathogen in the soil and the devastating effects of the disease. Use of the biorational products developed in this project could be an effective and environmentally compatible way for managing bacterial wilt and helping to restore the productivity in infested land used for this important cash crop. Furthermore, it will help to make the most use of nonrenewable resources. In addition, implementation of natural plant extracts and biological control agents is a focus of this project, which will meet the requirement of sustainable agriculture to integrate or make the most use of natural biological controls. Management of bacterial wilt and bacterial spot on tomatoes has relied primarily on conventional disease control measures, especially conventional pesticides such as copper bactericides for bacterial spot and methyl bromide/chloropicrin for bacterial wilt. Not only do these chemicals not provide satisfactory disease control, but they also pose threats to the environment (copper toxicity, influence of methyl bromide/chloropicrin on the ozone layer and potential adverse effect of these chemicals on beneficial microorganisms in nature). Development of biorational approaches in the proposed project will significantly enhance soil and water quality and human health. The products and techniques developed in this proposal are environmentally-compatible and grower-friendly. In comparison to the intensive use of conventional chemical pesticides, use of the reduced risk tactics proposed in this project will reduce threats to growers, reduce pollution to their land, food and water resources, and will enhance the quality of life for farmers and consumers.

Objectives/Performance Targets

1. To develop strategies in integrated management of bacterial wilt: a. Evaluate the efficacy and application methods of new biofumigants and reduced risk compounds in control of R. solanacearum on tomato under greenhouse and field conditions. b. Determine efficacy of the SAR inducer, Actigard, in reducing bacterial wilt on susceptible tomato cultivars under field conditions at different inoculum levels, and evaluate integrated effectiveness and economics of field application of Actigard, biofumigant (thymol), and commercial tolerant or resistant tomato genotype (FL 7514, BHN 669) in the management of bacterial wilt. c. Using the data obtained in objectives 1a and 1b to develop and implement best management strategies for bacterial wilt on tomato in naturally infested commercial tomato fields. On-farm research and demonstrations will be conducted in collaboration with growers and extension agents in north Florida and southern Georgia. 2. To optimize integrated management of bacterial spot with the SAR inducer Actigard, PGPRs and bacteriophages. a. Determine if lower rates of Actigard can be applied to enhance disease control without affecting tomato yield and identify resistant lines to determine if they respond to PGPRs. b. Determine the effects of modified application strategies of the SAR inducer (Actigard) and PGPRs in combination with bacteriophages. c. Combine the best strategies in 2a and 2b for management of bacterial spot in field experiments to achieve maximum reduction of the disease and copper bactericide application. On-farm research and demonstration will be conducted in north Florida and southern Georgia and economic benefits will be analyzed. 3. To determine, through Cost Benefit Analysis of each field trial, the management strategies yielding the greatest financial returns to the grower.

Materials and Methods

Objective 1: Integrated Management of Bacterial Wilt a. Evaluate the efficacy and application methods of new biofumigants and reduced risk compounds in control of R. solanacearum on tomato under greenhouse and field conditions. These experiments will be conducted at NFREC, Quincy, FL. Effectiveness of several biological or plant-derived products, including Muscodor, carvacrol, geraniol and linalool, as biofumigants as shown in in vitro studies for control of R. solanacearum will be evaluated in greenhouse studies. Soil will be infested with R. solanacearum. Varying rates of these compounds will be used to fumigate infested soils in closed plastic bags. Treated soils will be transferred to 10-cm pots and seedlings of a susceptible tomato cultivar will be transplanted into the pots. Thymol, which was shown to be a promising biofumigant for bacterial wilt control in our previous studies, will be used as a control. Infested soils not fumigated with these compounds will be used as the untreated control. Bacterial wilt incidence will be recorded until four weeks after transplanting. Yucca extracts and Serenade, two commercial products containing primarily natural or reduced-risk components, will be tested to treat soils infested with R. solanacearum. Effective application dosage of these products will be determined. Field trials to evaluate application methods and efficacy of biofumigants and other products with promising disease control in greenhouse studies will be conducted in the fall of 2006 and 2007. Field soil will be uniformly infested with the bacterial pathogen. After preparing beds a single drip line is normally laid which is followed by laying of plastic mulch. However, for this experiment, one additional drip line will be laid in plots where stable solutions of these products are to be applied. The biofumigants and other products will be applied in the soil by drip irrigation for 3 h. Three days after application, holes will be pierced through the plastic mulch for transplanting and to allow aeration of excess volatiles. The period of aeration will be 1 week. Muscodor will not be applied through drip irrigation but broadcast and incorporated into the soil. Tomato seedlings susceptible to bacterial wilt will be transplanted into the field seven days after application of Muscodor. Thymol will be tested in the field to compare its efficacy with these products. These field experiments will have thymol and other compounds, and untreated control. Bacterial wilt incidence will be recorded bi-weekly starting 10 d after transplanting and the AUDPC will be calculated and yield data will be collected. b. Determine efficacy of the SAR inducer, Actigard, in reducing bacterial wilt on susceptible tomato cultivars under field conditions at different inoculum levels, and evaluate integrated effectiveness and economics of field application of Actigard, biofumigant (thymol), and commercial tolerant or resistant tomato genotype (FL 7514, BHN 669) in the management of bacterial wilt. These field experiments will be conducted at NFREC, Quincy, FL. Field experiments to determine the effect of Actigard against bacterial wilt on commercial susceptible cultivars will be conducted in fall 2006 and 2007. Tomato cultivars FL 47 and Solar Set will be used, and a resistant cultivar FL 7514 will also be tested to compare effects. Seeds will be sown in polystyrene flats. Transplants will be sprayed with the SAR compound Actigard at the rate of 5 g per 100 L 2 wk after seedling emergence and 5 d before transplanting. Field soil will be infested with R. solanacearum uniformly with different inoculum concentrations. Post-transplant Actigard applications will be at the rate of 5 g per 100 L. A total of six foliar applications will be made once every week in the field. Bacterial wilt incidence will be recorded bi-weekly starting 10 d after transplanting until the end of the season and yield data will be collected. The integrated effect of Actigard, thymol and resistant tomato genotypes in control of bacterial wilt will be evaluated in the meantime. Two commercially available resistant cultivars, FL 7514 and BHN 669, will be included in the studies and a susceptible cultivar will be used as a control. Field soil will be infested with R. solanacearum artificially. Thymol solution will be applied to the field through a drip line for 3 h. Following fumigation for 3 d under the plastic mulch and 1 wk for aeration to vent excess volatiles, tomato seedlings that are susceptible and resistant to bacterial wilt will be transplanted. These field experiments will have thymol, Actigard, thymol plus Actigard (i.e, integrated application of thymol and Actigard), and untreated controls as main plots, and different tomato cultivars as split plots. Timing and dosage of pre- and post-transplant Actigard applications will be the same as mentioned above. Bacterial wilt incidence will be recorded bi-weekly starting 10 d after transplanting until the end of the season and yield data will be collected. c. Use the data obtained in objectives 1a and 1b to develop and implement best management strategies for bacterial wilt on tomato in naturally infested commercial tomato fields. On-farm research and demonstrations will be conducted in collaboration with growers and extension agents in north Florida and southern Georgia. The most promising findings from all experiments conducted in 2006 and 2007 will be used to conduct on-farm studies and demonstrations in 2008. The on-farm studies and demonstrations will be carried out in collaboration with Lester Muralles and Joel Hudgins, county extension agents in north Florida and south Georgia, respectively, and Thomas Smith and Greg Murray, tomato growers in Quincy, Florida and Bainbridge, Georgia. Each field plot will be 150 ft in length and contain 6 rows. For example, the biofumigant thymol and other promising reduced risk products will be used as pre-plant treatments to reduce or eliminate initial inoculum of R. solanacearum in infested soils, and Actigard will be used to enhance resistance of tomato plants against the disease prior to and after transplanting into the field. Tolerant cultivars can be combined with application of Actigard and/or application of thymol or other biorational products. Conventional standard measures used by the growers will be used as a control. Because of the herbicidal activity of thymol, effect of thymol treatment on common and dominant weeds in tomato field, especially nutsedge, will be evaluated. Incidence of bacterial spot will be monitored, and incidence of diseases caused by soil-borne fungal pathogens, including Sclerotinia, Fusarium, Pythium and Rhizoctonia species, will also be monitored to evaluate fungicidal effect of thymol. Objective 2: Integrated Management of Bacterial Spot a. Since SAR inducers appear to have limited effects on plant yield, we will determine if lower rates of Actigard can be applied to enhance disease control without affecting yield and identify resistant lines to determine if they respond to PGPRs. The experiments will be conducted under greenhouse conditions. Seeds from Bonny Best, a susceptible genotype and tomato genotypes, PI 114490 and 684490 (the latter is derived from crosses between PI 1684 and PI 114490 developed by R. E. Stall and J. B. Jones) with high levels of resistance to tomato race 2 and moderate levels of resistance to tomato races 1 and 3 derived from crosses made with PI114490 will be tested for activity with reduced rates of Actigard. Seed will be planted in 10-cm pots containing a soilless medium. Seedlings will be grown to the four-leaf stage and then treated with Actigard. Actigard will be applied at 1x, 1/2x and 1/10x label rate. Applications will be made two weeks and again 4 days prior to inoculation. The plants will be inoculated with the tomato race 1 and tomato race 3 pathogens, placed in polyethylene bags in a growth chamber and incubated at 21C to 24C for 36 hr. The plants will be returned to the greenhouse bench. Disease severity will be assessed approximately 14 to 21 days after inoculation. The tomato genotypes will also be tested to determine if they respond to PGPRs. Seeds will be infested with either bacterial suspensions of the PGPRs (strains 89-B61 and SE34) in sterile saline (0.85% sodium chloride) or with sterile saline solution as a control. Seedlings will be grown to the four-leaf stage and then treated a second time with the PGPRs. One week after the second application of the PGPR suspension, the plants will be inoculated with the pathogens. In separate experiments, the plants will be inoculated with a strain of the bacterial spot pathogen tomato race 1 or tomato race 3, placed in polyethylene bags and then placed in a growth chamber at 21C for 36 hr. The plants will be returned to the greenhouse mist chamber for 36 h and then returned to the greenhouse bench. Disease severity will be assessed approximately 21 days after inoculation. b. Determine the effect of modified application strategies of SAR inducers (Actigard) and PGPRs (SE34 and 89-B61) in combination with bacteriophages. In greenhouse studies, seeds from tomato genotypes with high levels of resistance to bacterial spot identified in objective 2a that respond to low levels of Actigard will be planted in 10-cm pots containing a soilless medium. Actigard will be applied at 1x, 1/2x and 1/10x label rate. Applications will be made two weeks and again 4 days prior to inoculation. For treatment with PGPRs (strains 89-B61 and SE34), seeds from tomato genotypes with high levels of resistance to tomato race 2 and moderate levels of resistance to tomato races 1 and 3 will be infested with either a suspension of the PGPRs in sterile saline (0.85% sodium chloride) or with sterile saline solution. Seedlings will be grown to the four-leaf stage and then treated a second time with the PGPRs one week prior to inoculation. The bacteriophage will be applied immediately prior to inoculation. The plants will be inoculated with bacterial spot pathogen tomato race 1, tomato race 3, placed in a polyethylene bag and then placed in a growth chamber at 24C for bacterial spot for 36 hr. The plants will be returned to the greenhouse mist chamber for 36 h and then returned to the greenhouse bench. Disease severity will be assessed approximately 21 days after inoculation. In an attempt to determine any interactions between treatments for controlling Xcv tomato races 1 and 3, greenhouse experiments will be conducted with select tomato genotypes and will include the following foliar treatments with or without seed/root treatment using PGPR: 1) copper/Mancozeb; 2) bacteriophage or Actigard (1/x, 1/2x or 1/10x); 3) bacteriophage + Actigard (1/x, 1/2x or 1/10x); 4) 89-B61 + bacteriophage or Actigard (1/x, 1/2x or 1/10x); 5) 89-B61 + bacteriophage + Actigard (1/x, 1/2x or 1/10x); 6) SE34 + bacteriophage or Actigard (1/x, 1/2x or 1/10x); 7) SE34 + bacteriophage + Actigard (1/x, 1/2x or 1/10x). c. Combine the best management strategies in 2a and 2b for bacterial spot in field experiments to achieve maximum reduction of the disease and copper bactericide application. On-farm research and demonstration will be conducted in north Florida and southern Georgia. Seeds from tomato breeding lines identified in objective 2a with resistance to bacterial spot tomato race 2 (T2) will be planted in Speedling trays in soilless potting mix and grown in the greenhouse. For treatment with PGPRs, seeds from tomato genotypes with high levels of resistance to Xcv tomato race 2 and moderate levels of resistance to Xcv tomato races 1 and 3 will be infested with either suspensions (108 CFU/ml) of the PGPRs in sterile saline (0.85% sodium chloride) or with sterile saline solution. Seedlings will be grown to the four-leaf stage and then treated a second time with the PGPRs. One seedling in the center of each tray will be inoculated by infiltrating a leaflet with a suspension of the bacterial spot pathogen T3. Prior to transplanting the plants will be inoculated with strains of races 1 and 3 of the bacterial spot pathogen in the fall of 2007 and 2008. The foliar treatments will be applied in the greenhouse as follows: bacteriophage will be applied in every irrigation; SAR compounds will be applied at 1x, 1/2x and 1/10x label rate once per week beginning in the greenhouse. When the transplants are set in the field in a randomized complete block design, the bacteriophage applications will be applied twice per week immediately before sunset. The SAR compounds will be applied once every two weeks, and the standard copper/mancozeb treatment will be applied once every week. In order to compare the effects of copper and non-copper foliar treatments on disease control, the following will be included in the field experiments: 1) untreated control; 2) copper applied once a week (20 lb/acre per season); 3) phage/Actigard + copper (copper applied once a week, i.e. 20 lb/acre per season); 4) phage/Actigard + copper (copper applied once every two weeks, i.e. 10 lb/acre per season); 5) phage/Actigard + copper (copper applied once every three weeks, i.e. 6.7 lb/acre per season); 6) phage/Actigard. Calculation of total copper application is based on 10 weeks in a growing season. Rate of copper bactericide will be 2 lb/acre per application. Mancozeb will be used along with copper bactericides at 2.5 lb/acre per application. Phages will be applied twice every week and Actigard once every two weeks. Disease ratings will be made by assessing percent defoliation at several time periods and then the area under the disease progress curve (AUDPC) will be calculated. On-farm studies and demonstrations will be conducted in fall of 2008 in two farms located in north Florida and southern Georgia. Applications with biological control agents (bacteriophages and PGPRs) and Actigard will be compared to standard copper/Mancozeb treatment. Objective 3: To determine, through Cost Benefit Analysis of each field trial, the management strategies yielding the greatest financial return to the grower.

Cost/benefit analyses will be conducted, using yield and cost data collected from field trials for each management strategy selected (Objectives 1c and 2c), to quantify the incremental economic value of the identified IPM strategies relative to standard grower practices. A partial budget approach will be used to determine whether the change in benefits, as a result of the adoption of the new sustainable strategy, exceeds the change in program costs. Net returns will be used to quantify the immediate financial benefits of each new tactic. An analysis of additional capital requirements will be conducted to determine if the additional capital investment required by the new management program will generate sufficient income to cover added capital costs.

Results and Discussion/Milestones

Objective 1. Field experiment 2006. Typical bacterial wilt symptoms were observed as early as 1 week post transplanting. Wilted plants were assayed for Ralstonia by first performing a bacterial ooze test, and then followed by using the immunoassay strips assay or by FAME. All the plants that were sampled were positive for the presence of the bacterium. In all the experiments the susceptible cultivar, Phoenix, was affected most by the pathogen; by the end of the experiment the Phoenix plants in the UTC produced the least amount of fruit compared to the 2 resistant cultivars, BHN669 and FL7514 (Table 1). Phoenix plants that received thymol or thymol and acibenzolar-S-methyl treatments had more than a 200-fold increase in fruit production, and a 3-fold decrease of plants wilting for thymol and an almost 5-fold for thymol and acibenzolar-S-methyl. The UTC plants had 94% of the plants wilt by the end of the experiment, while Phoenix plants treated with thymol or thymol and acibenzolar-S-methyl had 30% and 19% respectively (data not shown). The moderately resistant cultivars experienced a significant increase in marketable yield and significant decrease of plants wilting. For each cultivar there was a significant difference between the UTC and treated plants. A significant difference was determined for moderately resistant plants treated with thymol or thymol and acibenzolar-S-methyl when compared to the UTC when contrasting disease incidence (Table 1). Plants treated with thymol and acibenzolar-S-methyl resulted over 70% increase in marketable yield and at a 30% reduction of disease incidence for all 3 cultivars. Field experiment 2008. Typical bacterial wilt symptoms were observed as early as the first week post transplanting. Every wilted plant that was tested was positive for Ralstonia. Testing consisted of the bacterial ooze test and then confirmed by using immunoassay strips. In this experiment the Phoenix cultivar survived better than FL7514, the moderately resistant cultivar; might be due to the amount of rain received from the hurricane. Regardless of the differences between the two cultivars, plants treated with thymol, acibenzolar-S-methyl, or thymol and actigard had greater yields and less wilted plants than the untreated controls for both cultivars (Table 2). A significant difference was noted for the thymol and acibenzolar-S-methyl treatment than the UTC, thymol, or acibenzolar-S-methyl treatments for the moderately resistant cultivar when comparing the disease incidence standard errors (Table 2). Previously, we determined that the use of thymol and acibenzolar-S-methyl in field conditions was able to decrease disease incidence and increase fruit yield (Ji et al., 2005; Pradhanang et al., 2005). This study was the first time the application of thymol and acibenzolar-S-methyl was used together in field conditions to control bacterial wilt on moderately resistant tomato cultivars. We report that the use of both products will not have a negative affect on the tomato production. The combination of both products numerically increased the fruit yield and decreased the disease incidence for the susceptible cultivar. In both trials the moderately resistant plants that received the thymol, acibenzolar-S-methyl, the combination of both chemicals increased fruit yield and the lower disease incidence when compared to the UTC. In both studies the combination of thymol and acibenzolar-S-methyl was significantly different than the UTC, thymol or acibenzolar-S-methyl treatments, when comparing disease incidence on moderately resistant cultivars. Thus in agreement with what Pradhanang et al. (2005) reported, we observed a greater difference in disease incidence on resistant plants than susceptible plants treated with acibenzolar-S-methyl. Susceptible tomato cultivars treated with acibenzolar-S-methyl were resistant to the pathogen only when the bacterial populations were low, 105-106; acibenzolar-S-methyl was determined ineffective in increasing resistance when the pathogen populations were 107 or higher (Anith, 2004; Pradhanang, 2005). Controlling bacterial wilt in field conditions has been studied for decades (Kelman, 1953), and to date no single strategy has proven to be effective for reducing disease incidence or the severity of the symptoms (Denny, 2006). Factors such as the pathogen's ability to colonize alternative hosts (Hong, 2008), the longevity of the bacterium in fallow soil and water (Hayward, 1991), and its ability to persist in infested plant debris (Granada, 1983), have made it difficult to control the disease once it has become established in the field. Good cultural practices also referred to as Integrated Disease Management (IDM) encompasses multiple strategies for controlling the disease. Included in IDM is avoiding planting in pathogen infested soil with pathogen free crops, irrigating with pathogen free water, and proper sanitation practices of operation tools, which are all important to exclude or reduce the pathogen (Anith et al., 2004; Champoiseau, 2009; Hong, 2008; Denny 2006). Complete resistance is only found in groundnut, but semi-resistant cultivars are available, however resistance is limited to geographical location (Denny, 2006). With the decreased use of methyl bromide, alternatives to control soil pathogens have been increasingly studied (Martin, 2003; Noling, 1994; Santos, 2006). Thymol has proven to be effective in controlling pests such as fungi, nematodes, insects, and bacteria (Delespaul, 2000; Ji, 2005; Lee, 1997; Šegvić, 2006). Acibenzolar-S-methyl too has been proven to be effective against soil borne fungi, nematodes, and bacteria (Benhamou and Bélanger, 1998; Chinnasri, 2003; Pradhanang et al, 2005). With thymol's ability to control various pathogens, further research could determine its use as a methyl bromide alternative. Again, it is recommend using moderately resistant cultivars to lower disease incidence and for maximum yield. We showed that if a grower were to use both chemicals, neither would be detrimental to yield production. As shown in before mentioned studies, both products are effective at decreasing the incidence of different plant diseases. Thus, the combination of both products could offer a wider protection against multiple biological inhibitory factors. Further studies would include determining the minimum inhibitory concentration (MIC) in field conditions for the most effective and economical benefit for the growers. In conjunction with determining the MIC, further studied of the effect of the combination of thymol and acibenzolar-S-methyl would have on other plant pathogens or on multiple diseases. Further research would also need to be conducted to determine the plant's responses to the chemicals and grafting, as grafting could be a new method for controlling the disease. Objective 1. Field experiment 2006. Typical bacterial wilt symptoms were observed as early as 1 week post transplanting. Wilted plants were assayed for Ralstonia by first performing a bacterial ooze test, and then followed by using the immunoassay strips assay or by FAME. All the plants that were sampled were positive for the presence of the bacterium. In all the experiments the susceptible cultivar, Phoenix, was affected most by the pathogen; by the end of the experiment the Phoenix plants in the UTC produced the least amount of fruit compared to the 2 resistant cultivars, BHN669 and FL7514 (Table 1). Phoenix plants that received thymol or thymol and acibenzolar-S-methyl treatments had more than a 200-fold increase in fruit production, and a 3-fold decrease of plants wilting for thymol and an almost 5-fold for thymol and acibenzolar-S-methyl. The UTC plants had 94% of the plants wilt by the end of the experiment, while Phoenix plants treated with thymol or thymol and acibenzolar-S-methyl had 30% and 19% respectively (data not shown). The moderately resistant cultivars experienced a significant increase in marketable yield and significant decrease of plants wilting. For each cultivar there was a significant difference between the UTC and treated plants. A significant difference was determined for moderately resistant plants treated with thymol or thymol and acibenzolar-S-methyl when compared to the UTC when contrasting disease incidence (Table 1). Plants treated with thymol and acibenzolar-S-methyl resulted over 70% increase in marketable yield and at a 30% reduction of disease incidence for all 3 cultivars. Field experiment 2008. Typical bacterial wilt symptoms were observed as early as the first week post transplanting. Every wilted plant that was tested was positive for Ralstonia. Testing consisted of the bacterial ooze test and then confirmed by using immunoassay strips. In this experiment the Phoenix cultivar survived better than FL7514, the moderately resistant cultivar; might be due to the amount of rain received from the hurricane. Regardless of the differences between the two cultivars, plants treated with thymol, acibenzolar-S-methyl, or thymol and actigard had greater yields and less wilted plants than the untreated controls for both cultivars (Table 2). A significant difference was noted for the thymol and acibenzolar-S-methyl treatment than the UTC, thymol, or acibenzolar-S-methyl treatments for the moderately resistant cultivar when comparing the disease incidence standard errors (Table 2). Previously, we determined that the use of thymol and acibenzolar-S-methyl in field conditions was able to decrease disease incidence and increase fruit yield (Ji et al., 2005; Pradhanang et al., 2005). This study was the first time the application of thymol and acibenzolar-S-methyl was used together in field conditions to control bacterial wilt on moderately resistant tomato cultivars. We report that the use of both products will not have a negative affect on the tomato production. The combination of both products numerically increased the fruit yield and decreased the disease incidence for the susceptible cultivar. In both trials the moderately resistant plants that received the thymol, acibenzolar-S-methyl, the combination of both chemicals increased fruit yield and the lower disease incidence when compared to the UTC. In both studies the combination of thymol and acibenzolar-S-methyl was significantly different than the UTC, thymol or acibenzolar-S-methyl treatments, when comparing disease incidence on moderately resistant cultivars. Thus in agreement with what Pradhanang et al. (2005) reported, we observed a greater difference in disease incidence on resistant plants than susceptible plants treated with acibenzolar-S-methyl. Susceptible tomato cultivars treated with acibenzolar-S-methyl were resistant to the pathogen only when the bacterial populations were low, 105-106; acibenzolar-S-methyl was determined ineffective in increasing resistance when the pathogen populations were 107 or higher (Anith, 2004; Pradhanang, 2005). Controlling bacterial wilt in field conditions has been studied for decades (Kelman, 1953), and to date no single strategy has proven to be effective for reducing disease incidence or the severity of the symptoms (Denny, 2006). Factors such as the pathogen's ability to colonize alternative hosts (Hong, 2008), the longevity of the bacterium in fallow soil and water (Hayward, 1991), and its ability to persist in infested plant debris (Granada, 1983), have made it difficult to control the disease once it has become established in the field. Good cultural practices also referred to as Integrated Disease Management (IDM) encompasses multiple strategies for controlling the disease. Included in IDM is avoiding planting in pathogen infested soil with pathogen free crops, irrigating with pathogen free water, and proper sanitation practices of operation tools, which are all important to exclude or reduce the pathogen (Anith et al., 2004; Champoiseau, 2009; Hong, 2008; Denny 2006). Complete resistance is only found in groundnut, but semi-resistant cultivars are available, however resistance is limited to geographical location (Denny, 2006). With the decreased use of methyl bromide, alternatives to control soil pathogens have been increasingly studied (Martin, 2003; Noling, 1994; Santos, 2006). Thymol has proven to be effective in controlling pests such as fungi, nematodes, insects, and bacteria (Delespaul, 2000; Ji, 2005; Lee, 1997; Šegvić, 2006). Acibenzolar-S-methyl too has been proven to be effective against soil borne fungi, nematodes, and bacteria (Benhamou and Bélanger, 1998; Chinnasri, 2003; Pradhanang et al, 2005). With thymol's ability to control various pathogens, further research could determine its use as a methyl bromide alternative. Again, it is recommend using moderately resistant cultivars to lower disease incidence and for maximum yield. We showed that if a grower were to use both chemicals, neither would be detrimental to yield production. As shown in before mentioned studies, both products are effective at decreasing the incidence of different plant diseases. Thus, the combination of both products could offer a wider protection against multiple biological inhibitory factors. Further studies would include determining the minimum inhibitory concentration (MIC) in field conditions for the most effective and economical benefit for the growers. In conjunction with determining the MIC, further studied of the effect of the combination of thymol and acibenzolar-S-methyl would have on other plant pathogens or on multiple diseases. Further research would also need to be conducted to determine the plant's responses to the chemicals and grafting, as grafting could be a new method for controlling the disease. Objective 2A-C (continued) The objectives of these experiments were to 1) test if Phosphorous acid salts (PAS) alone or in combination with standard copper-bactericide was effective in controlling bacterial spot of tomato caused by X. perforans under greenhouse conditions; 2) investigate the antibacterial property of PAS on X. perforans in vitro; and 3) test if PAS alone or in combination with standard copper-bactericide or ASM was effective in controlling bacterial spot of tomato caused by X. perforans under field conditions. In fall 2005 in Quincy, FL PAS treatment, either alone or mixed with copper-mancozeb, significantly suppressed bacterial spot disease development (Table 7). These treatments were as effective as the copper-mancozeb plus ASM for disease control. However, while the copper-mancozeb plus ASM significantly increased tomato yield, the two PAS treatments did not (data not shown). In fall 2006 in Quincy, the combination of PAS and copper-mancozeb significantly reduced disease development compared to the untreated control, whereas copper-mancozeb alone or in combination with ASM did not (Table 3). Tomato yield was not affected by any of the treatments (data not shown). In the spring 2007 in Quincy field 1, both PAS and PAS plus ASM treatments significantly reduced disease development compared to the untreated control (Table 7). However, in Citra field 1, none of the treatments contributed to significant disease reduction compared to the untreated control. In Quincy field 2, all treatments reduced disease development significantly compared to untreated control (Table 7). PAS combined with full rate or half rate of copper-mancozeb was significantly better in disease reduction than PAS alone. PAS combined with full rate of copper-mancozeb was not significantly different from PAS combined with half rate copper-mancozeb though full rate copper-mancozeb alone was significantly better than the half rate. However in Citra, a different phenomenon was observed. Both the full and half rates of copper-mancozeb reduced disease significantly, as did PAS combined with full rate copper-mancozeb. Nevertheless, treatments with PAS alone and in combination with half rate of copper-mancozeb were not significantly different from untreated control. Treatment with PAS alone was not significantly differently from copper-mancozeb, nor was it different from PAS combined with a half rate copper-mancozeb. In the fall trials in Citra, PAS alone and in combination with either full rate copper-mancozeb or ASM significantly reduced disease development compared to the UTC, whereas half rate copper-mancozeb and PAS combined with half rate copper-mancozeb did not. PAS alone, PAS plus ASM, and phosphorus acid plus full rate copper-mancozeb were as effective as the copper-mancozeb treatment in disease reduction. Our studies demonstrated that PAS alone or combined either with ASM or the standard copper-bactericide program significantly reduced bacterial spot disease severity on tomatoes (two cultivars: FL 47and Bella Rosa) in two locations in Florida in 3 years of field trials (total 7 field trials) without affecting yield. Weekly application of PAS alone obtained disease control in 4 out of 6 field trials compared to the untreated control, and was as effective as the copper-mancozeb standard in terms of disease control in 2 out of 6 field experiments. Weekly application of PAS combined with the standard copper-bactericide program (full rate) provided disease control as effectively as the standard copper bactericide program consistently. Weekly PAS plus biweekly ASM application was as effective as the standard copper-bactericide program in disease control in 2 of 3 field trials without impact on yield. The effect of PAS when alternated with the standard copper-bactericide program was similar to the copper-mancozeb plus ASM; however it was only tested in one field trial. More field experiments are needed to confirm that effect before it could be recommended to tomato growers. Based on these experiments, weekly application of PAS at (2.3-4.6 L/ha) plus the standard bactericide program or weekly application of PAS at (2.3-4.6 L/ha) plus biweekly ASM (54 mg/L) proved to be effective new strategies for tomato growers to manage bacterial spot of tomato in the field in North and Central Florida where race 3 or 4 of Xanthomonas perforans is predominant.

Impact of Results/Outcomes

Growers are becoming more aware that management of bacterial wilt and bacterial spot on tomatoes will require changes rather than continuing to rely primarily on conventional disease control measures, especially conventional pesticides such as copper bactericides for bacterial spot and methyl bromide/chloropicrin for bacterial wilt. Not only do these chemicals not provide satisfactory disease control, but they also pose threats to the environment (copper toxicity, influence of methyl bromide/chloropicrin on the ozone layer and potential adverse effect of these chemicals on beneficial microorganisms in nature). Development of biorational approaches in the proposed project will significantly enhance soil and water quality and human health. The products and techniques developed in this proposal are environmentally-compatible and grower-friendly. In comparison to the intensive use of conventional chemical pesticides, use of the reduced risk tactics proposed in this project will reduce threats to growers, reduce pollution to their land, food and water resources, and will enhance the quality of life for farmers and consumers.

Economic Analysis

We did not address this issue.

Publications/Outreach

Wen, A., Botond Balogh, M. Timur Momol, Stephen M. Olson, and Jeffrey B. Jones, 2009. Management of bacterial spot of tomato with phosphorous acid salts. Crop Protection 28: 859-863. Wen, A., Botond Balogh, M. Timur Momol, Stephen M. Olson, and Jeffrey B. Jones, 2008. Control of bacterial spot of tomato with a phosphorous acid product. Phytopathology 98:S169. Presented at the 2008 American Phytopathological Society meetings. Jason C. Hong, M. Timur Momol, Pingsheng Ji, Stephen M. Olson, James Colee and, Jeffrey B. Jones. Combination of Thymol and Acibenzolar-S-Methyl to Aid in Controlling Bacterial Wilt in Tomato at Field Conditions. (To be submitted as refereed article to Crop Protection).

Farmer Adoption

One grower is manager for a large tomato production operation in North Florida. They normally have about 700 acres split between spring and fall. They had used copper based materials for years but as a result of our work they have not used any copper based materials for last 2 years (4 crops). They have been using Actigard, phage and Tanos. Tanos is also being used to help suppress Target spot. We also have a number of other growers in the north Florida/south Georgia area that use Actigard and phage to help manage BS, but they have not dropped out copper all together but have greatly reduced it’s use. Other growers are using alternative methods for bacterial spot control based on work from our program.

Areas Needing Additional Study

Clearly more work is necessary for finding improved strategies for disease control of bacterial spot and bacterial wilt; however, we do provide information for improved control strategies that in some cases growers are beginning to accept. Plant activators continue to provide significant disease control; however, there are reduced yield responses from application of activators which requires further study in order to determine what can be done to reduce this as a problem. Bacteriophages have shown considerable use in one of the experiments. However, we had several experiments ruined by viral disease and/or excessive rain so that further experimentation was unsuccessful.

Participants:

Joel Hudgins
County Extension Director
University of Georgia
Decatur County Extension, 1401 Loblolly Lane
Bainbridge, GA 39817
229-248-3033
E-mail: jhudgins@uga.edu

Pingsheng Ji
Research Associate
University of Florida
North Florida Research and Education Center, 155 Research Road
Quincy, FL 32351
850-875-7155
E-mail: pji@ufl.edu

Tim Momol
University of Florida
University of Florida, PlantPathology Department
Gainesville, FL 32611
352-392-1781
E-mail: tmomol@fl.edu

Lester Muralles
County Extension Agent
University of Florida
Gadsden County Extension, 2140 W. Jefferson Street
Quincy, FL 32351
850-875-7255

Greg Murray
Tomato grower
Dale & Greg Murray Farms
3654 Faceville Hwy
Bainbridge, GA 31717
229-246-6563

Steven Olson
Professor
University of Florida
North Florida Research and Education Center, 155 Research Road
Quincy, FL 32351
850-875-7144
E-mail: smolson@ifas.ufl.edu

John Smith
Senior Statistician
University of Florida
North Florida Research and Education Center, 155 Research Road
Quincy, FL 32351
850-875-7141
E-mail: JLSmith@ifas.ufl.edu

Thomas Smith
Tomato grower
Thomas Smith Farm
9540 Flat Creek Road
Quincy, FL 32351
850-567-9998

Attachments:

Tables: http://mysare.sare.org/mySARE/assocfiles/921222Final Sare report 2010 Tables.pdf
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