Application of Acibenzolar-S-Methyl Enhances Host Resistance in Tomato Against *Ralstonia solanacearum*

P. M. Pradhanang, P. Ji, M. T. Momol, and S. M. Olson, North Florida Research and Education Center, University of Florida, IFAS, 155 Research Road, Quincy 32351; J. L. Mayfield, Gadsden County Extension, University of Florida, IFAS, Quincy 32351; and J. B. Jones, Plant Pathology Department, 2253 Fifield Hall, University of Florida, IFAS, Gainesville 32611

ABSTRACT

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 Dis. 89:989-993.

The chemical elicitor acibenzolar-S-methyl (ASM; Actigard 50 WG), which induces systemic acquired resistance (SAR), was investigated to determine the effect on bacterial wilt of tomato caused by *Ralstonia solanacearum* on moderately resistant cultivars under greenhouse and field conditions. In greenhouse experiments, ASM was applied as foliar spray and/or soil drench (3 µg/ml) before and as foliar spray (30 µg/ml) after transplanting. The chemical elicitor was ineffective in reducing bacterial wilt incidence on susceptible tomato cultivars Equinox and FL 47 when plants were inoculated with *R. solanacearum*. However, greenhouse studies indicated that ASM significantly enhanced resistance in cultivars with moderate resistance to bacterial wilt such as Neptune and BHN 466. It appeared that ASM-mediated resistance was partially due to prevention of internal spread of *R. solanacearum* toward upper stem tissues of tomato plants. The effect of ASM on moderately resistant cultivars was consistent in field experiments conducted in 2002 and 2003 in Quincy, FL, where bacterial wilt incidence was significantly reduced in ASM-treated BHN 466 (in 2002), FL 7514 (in 2003), and Neptune (both years) plants. ASM-treated BHN 466 and FL 7514 produced significantly higher tomato yield than the untreated controls. This is the first report of ASM-mediated control of bacterial wilt under field conditions, which suggests that use of this treatment for moderately resistant genotypes may be effective for control of bacterial wilt of tomato.

Additional keywords: induced resistance, latent infection

Bacterial wilt caused by *Ralstonia solanacearum* (race 1, biovar 1) is one of the most important diseases of tomato in the southeastern United States. The global importance of this disease is due to its destructive nature, wide host range, and geographical distribution of the pathogen. Integrated management strategies developed include the use of resistant cultivars, healthy transplants, and crop rotation with nonhost cover crops. Resistant tomato cultivars have been developed for fresh market production in the United States (32), but they are only moderately resistant to bacterial wilt and have not been widely adopted by growers. The Asian Vegetable Research and Development Center (AVRDC) has developed bacterial wilt resistant cultivars that are grown in some developing countries (25); however, their resistance is limited to locations, climate, strains of the pathogen, and soil character-

istics (14,37). Although crop rotation with nonhost crops may suppress soilborne populations of the pathogen (2,9,26,30), the pathogen survives in the environment in association with weed hosts, which impairs the effect of crop rotation (15,17,27,28). Plant-derived volatile essential oils such as thymol and palmarosa oil were effective biofumigants against *R. solanacearum* in greenhouse trials (29), but further field evaluation is necessary before their practical use for management of this disease is possible. Due to limited efficacy of current management strategies, bacterial wilt caused by *R. solanacearum* continues to be economically important in field-grown tomato production in the southeastern United States and many subtropical and tropical areas of the world.

The phenomenon in which a plant can activate protective mechanisms following contact by a pathogen or invader is termed systemic acquired resistance (SAR) (35). Recently, products called "plant activators" that induce SAR in plants were identified. One such compound, acibenzolar-S-methyl (ASM; Actigard 50 WG, Syngenta, Basel, Switzerland), has shown activity against several bacterial diseases including bacterial spot and bacterial speck of tomato (16,19,38) and fire blight of apples (22).

Application of ASM also reduced incidence of bacterial wilt in the susceptible tomato cultivar Solar Set when the plants were inoculated with low concentrations of *R. solanacearum* (10^5 or 10^6 CFU/ml) under greenhouse conditions (3). Induced resistance by ASM has also been reported in various plants for control of viral and fungal pathogens (5,7,12,20,23). ASM seems to have broad-spectrum functions in control of various plant diseases; however, the capability of ASM in the management of bacterial wilt of tomato on moderately resistant cultivars and its potential use under field conditions have not been studied.

The objective of this study was to investigate the effect of ASM on bacterial wilt incidence and yield of moderately resistant tomato cultivars under greenhouse and field conditions.

MATERIALS AND METHODS

Bacterial culture and inoculum preparation. *R. solanacearum* (race 1, biovar 1), tomato strain Rs5 (28) isolated in Quincy, FL, was used in these studies. The bacterial pathogen was grown at 28°C either on casamino acid peptone glucose (CPG) agar (peptone 10 g, casamino acids 1 g, dextrose 2.5 g, agar 15 g, deionized water 1 liter) for 48 h or in CPG broth on a shaker (200 rpm) for 18 h (overnight). Bacterial cells were suspended in sterile deionized water, and the concentration of inoculum was estimated by measuring absorbance at 590 nm. The viable bacterial population was determined following dilution plating on CPG agar. Tomato plants were inoculated with bacterial suspensions ranging from 1.2×10^7 to 2.6×10^8 CFU/ml.

Greenhouse experiments. Greenhouse experiments were conducted to determine the effect of ASM in controlling *R. solanacearum*. In preliminary experiments, ASM was tested on tomato cultivar BHN 466 (BHN Seeds, Bonita Spring, FL), which is moderately resistant to bacterial wilt. A susceptible cultivar Equinox was used as a control. In subsequent greenhouse assays, both Neptune (32) and BHN 466, which are moderately resistant to bacterial wilt, were included in addition to the susceptible cultivar Equinox.

Tomato seeds were sown in Terra-lite agricultural mix (Scott Sierra Horticultural Products Co., Marysville, OH), which will

Corresponding author: M. T. Momol
E-mail: TMOMOL@UFL.EDU

Accepted for publication 27 May 2005.

be referred to as soil, in expanded polystyrene flats with 2.5×2.5 cm cells. The chemical inducer ASM was applied as a foliar spray and soil drench. The initial foliar application of ASM was performed 2 weeks after seedling emergence, followed by a foliar and soil drench application 5 days prior to plant inoculation with the pathogen. For foliar sprays, a volume of 10 ml of ASM solution ($3 \mu\text{g/ml}$) was misted onto foliage of each plant using a hand-held garden sprayer. For soil drench, 5 ml of ASM solution at the same concentration was applied to each transplant cell. Tomato seedlings with three to four expanded leaves were inoculated by applying 5 ml of *R. solanacearum* suspension (1.2×10^7 and 2.6×10^8 CFU/ml in experiment 1 and experiment 2, respectively) into each transplant cell (33). Seedlings were transplanted into 10-cm pots 3 days after inoculation. Pots were placed in a saucer containing water to maintain high soil moisture in order to facilitate infection and wilt development. The plants were supplied with Peter's Peat Lite Special (15:16:17 N-P-K) at 10-day intervals at a rate of 7.5 g per liter of water. Tomato plants were maintained in a greenhouse with temperatures of 23 to 28°C during the night and 30 to 35°C for the day.

ASM was sprayed onto tomato foliage 4 days after transplanting and at weekly intervals thereafter at a concentration of 30 $\mu\text{g/ml}$, 20 ml each plant. Unless otherwise stated, a total of four foliar applications of ASM were made in the greenhouse after transplanting. Pots were arranged in randomized complete blocks with five replications. Each treatment was comprised of eight plants per replication. Bacterial wilt incidence was recorded weekly and quantified as the percentage of tomato plants wilted. Plant height was measured prior to wilting of individual plants over time, and the final measurement of plant height and bacterial wilt incidence was made 30 days after inoculation with *R. solanacearum*. Plants that did not develop wilt symptoms within 30 days after inoculation were tested for latent infection. Sections of basal stems (1 cm in length) were sterilized in 70% ethanol, washed with sterile water, and macerated in 1 ml of sterile deionized water as described previously (29). The macerates were streaked on modified SMSA agar, and the plates were incubated at 28°C. To make SMSA agar, filter-sterilized aqueous solutions of the following ingredients were added to 1 liter of the CPG medium: polymixin B sulfate 100 mg, bacitracin 25 mg, chloramphenicol 5 mg, penicillin 0.5 mg, 2,3,5 triphenyl tetrazolium chloride salts 50 mg, crystal violet 5 mg (10,11).

Upward movement of *R. solanacearum*. Greenhouse assays were conducted to determine the effect of ASM on movement of *R. solanacearum* in tomato plants prior to symptom development. ASM was

sprayed 2 weeks after seedling emergence and followed by a foliar and soil drench application 5 days before inoculation as described above for previous greenhouse experiments. Seedlings were transplanted into 10-cm pots 3 days after inoculation. In order to assess ASM effects on upward movement of *R. solanacearum* in tomato seedlings, basal and upper stem pieces of ASM-treated and untreated plants were processed for the bacterium 4 days after transplanting (7 days after inoculation) prior to symptom development. Sections of the basal and upper stem, each 1 cm in length, were taken from each plant at the collar level and 3 cm below the growing point, respectively. For each cultivar, stem sections from 50 ASM-treated and 50 untreated plants were surface-sterilized and macerated separately in 1 ml of sterile water, and a loopful of the macerated suspension was streaked on modified SMSA agar as described above. Seedlings of the tomato cultivars BHN 466 and Equinox treated with or without ASM application were analyzed. A randomized complete block design was used for the experiments with five replications, 10 plants for each treatment in one replication. The experiment was repeated twice under greenhouse conditions.

Field experiments. In 2002, the field experiment was conducted in Quincy using two moderately resistant tomato cultivars, BHN 466 and Neptune. Equinox was included as a susceptible control. Seeds were sown in expanded polystyrene flats as described earlier. ASM was first applied 2 weeks after seedling emergence, followed by a foliar spray and soil drench 5 days before inoculation at the same rate as in the greenhouse experiments. Seedlings were inoculated with 5 ml of bacterial suspension (1.4×10^7 CFU/ml) as described in greenhouse assays and then transplanted into the field 3 days later. Raised beds 10 cm high by 91 cm wide and centered 1.8 m apart were prepared for tomato growth. Beds were fumigated with methyl bromide (67%) and chloropicrin (33%) at a broadcast equivalent rate of 392 kg a.i./ha, fertilized with 218-67-218 kg/ha of N-P-K, and covered with polyethylene mulch. A drip irrigation line was established under the polyethylene mulch, and the plots were irrigated to field capacity by drip irrigation prior to tomato transplanting. Plots consisted of two rows 5 m long with 10 plants per row at 50-cm spacing within a row. Tomato seedlings were staked and sprayed with standard insecticides and fungicides weekly. Field applications of ASM (30 $\mu\text{g/ml}$) were made with a backpack CO_2 -powered sprayer adjusted to 60 psi and fitted with a boom to apply a 1-m band with approximately 30 or 55 ml sprayed on each plant depending on its age. The experiment was a randomized complete block design with a split plot arrangement for the cultivars and six replications. ASM applications were made at 7-

day intervals for 30 days following transplanting and at 15-day intervals for an additional 30 days. Bacterial wilt incidence was recorded at weekly intervals after transplanting and quantified as the percentage of plants wilted. Marketable yield of tomato fruits was recorded at harvest. In 2002, two harvests were made at the end of the season.

In 2003, a similar experiment was conducted in Quincy. Two moderately resistant tomato cultivars, Neptune and FL 7514 (Rupp Seeds Inc., Wauseon, OH), were used, and a susceptible cultivar, FL 47, was used as a control. FL 7514 is a commercially available cultivar derived from Neptune (J. W. Scott, *personal communications*). Tomato seedlings were sprayed with ASM 2 weeks after seedling emergence in a greenhouse, followed by a foliar and soil drench application 5 days prior to pathogen inoculation. For foliar sprays, a volume of 10 ml of ASM solution (25 $\mu\text{g/ml}$) was applied to each plant. For soil drenches, 5 ml of ASM suspension (12.5 $\mu\text{g/ml}$) was applied to each transplant cell. Five-week-old tomato seedlings were inoculated with the pathogen by delivering 5 ml of the bacterial suspension (6×10^7 CFU/ml) into each transplant cell and then transplanted into the field 3 days later. Preparation of beds for tomato growth used methods described above for the 2002 field trial. Six foliar sprays of ASM were applied in the field at intervals used in the field trial in 2002 but at a concentration of 25 $\mu\text{g/ml}$, approximately 30 or 55 ml per plant depending on plant age. A randomized complete block design was employed with split-plot arrangement for the cultivars. Each treatment was replicated six times with 17 plants in each replication. Bacterial wilt incidence was recorded at weekly intervals after transplanting. Tomato fruits were harvested at the end of the season. In 2003, a total of three harvests were made at weekly intervals. Marketable fruits were graded to USDA standards by a machine as extra large, large, and medium-sized fruit (34). The weight and number of fruits in each category were recorded and the average fruit size was calculated based on the weight and number of fruits in all size categories.

Statistical analysis. Chi-square (χ^2) tests were used to determine the effect of ASM on disease incidence, plant height, and movement of *R. solanacearum* in tomato plants under greenhouse conditions. Bacterial wilt incidence and tomato yield data from field experiments were analyzed using the ANOVA or GLM procedures of the Statistical Analysis System (SAS Institute, Cary, NC) for a split-plot randomized complete block design. An analysis of variance was conducted to determine the significance of main effects or interactions of Actigard and cultivars. Mean effects on each tomato cultivar were compared using Duncan's multiple range test.

RESULTS

Greenhouse experiments. In experiments carried out to evaluate the effect of ASM on bacterial wilt incidence, ASM application did not provide effective control of bacterial wilt on the susceptible cultivar Equinox (Table 1). However, bacterial wilt incidence in the treated moderately resistant cultivar Neptune was reduced compared with the untreated control. In experiment 1, none of the ASM-treated Neptune plants wilted, while 20% of untreated plants developed wilt symptoms (Table 1). In experiment 2, disease incidence of ASM-treated Neptune was significantly lower than that of the untreated control. ASM application also significantly reduced bacterial wilt incidence in the moderately resistant cultivar BHN 466 in one of the two experiments (Table 1).

Plant heights of ASM-treated BHN 466, Neptune, and Equinox were consistently higher than those of the untreated controls when all of the plants were inoculated with the pathogen, although this effect was not statistically significant (data not shown).

Effect of ASM on the distribution of *R. solanacearum* in stems. Upper and basal stem sections of ASM-treated and untreated BHN 466 and Equinox plants were processed for colonization by *R. solanacearum* 7 days after inoculation before wilt symptoms were observed. ASM significantly reduced colonization of the pathogen in upper stems of moderately resistant cultivar BHN 466, with 4 and 10% of upper stem sections infected in ASM-treated and untreated plants, respectively (Table 2). ASM application also showed a tendency to reduce basal stem infections on BHN 466. For the susceptible cultivar Equinox, the effect of ASM on bacterial movement in upper and basal stem tissues was significant (Table 2).

Field experiments. Tomato plants started wilting 7 days after transplanting into the field. In the 2002 trial, ASM application did not reduce bacterial wilt incidence on the susceptible cultivar Equinox (Table 3). The moderately resistant cultivars Neptune and BHN 466 exhibited partial wilt symptoms at early stages, but some plants recovered from expressing typical wilt symptoms and therefore kept growing. The difference in bacterial wilt incidence between ASM-treated and untreated plants of both Neptune and BHN 466 was significant (Table 3). ASM-treated BHN 466 produced 33% more fruit than untreated plants, which was statistically significant. Yield was 13% higher in ASM-treated Neptune than in the untreated control, although this difference was not significant (Table 3).

In the field trial of 2003, bacterial wilt incidence in ASM-treated moderately resistant cultivars Neptune and FL 7514 was significantly lower compared with the untreated controls (Table 4). The final

disease incidence of ASM-treated Neptune and FL 7514 was 10.8 and 18.6%, respectively, while it was 30.4 and 40.2%, respectively, in untreated plots. The two cultivars Neptune and FL 7514 did not exhibit significant differences in bacterial wilt resistance. Tomato yield of FL 7514 was significantly increased by ASM treatment, with an increase of 32.2% (Table 4). The yield of Neptune was also increased by 11.8% following ASM application, although this increase was not statistically significant. Application of ASM did not reduce disease incidence significantly in

the susceptible cultivar FL 47. FL 47 produced very low yields, irrespective of ASM application, due to high bacterial wilt incidence in ASM-treated and untreated plants (Table 4). A consistent numeric increase in average fruit size was achieved by ASM application in all three cultivars, although this increase was not statistically significant (data not shown).

DISCUSSION

The chemical elicitor ASM significantly reduced bacterial wilt incidence in moderately resistant cultivar Neptune and in two

Table 1. Effect of acibenzolar-*S*-methyl on percent bacterial wilt incidence in moderately resistant and susceptible tomato cultivars under greenhouse conditions

Treatment ^{x,y,z}	Experiment 1			Experiment 2		
	BHN 466	Neptune	Equinox	BHN 466	Neptune	Equinox
Actigard	5	0	95	15.6	40.6	100
Untreated	30	20	95	18.7	62.5	100
Chi-square (χ^2)	21.64 **	NA	7.14 NS	0.34 NS	9.60 **	NA

^x Acibenzolar-*S*-methyl (Actigard 50 WG) was applied twice before transplanting and four times after transplanting at weekly intervals.

^y BHN 466 and Neptune are moderately resistant to bacterial wilt, and Equinox is susceptible to the disease.

^z Mean of five replications. Values followed by ** indicate significant difference between Actigard treated and untreated plants for the corresponding cultivar at $P = 0.01$ based on χ^2 test. NS indicates not significant, and NA indicates chi-square test not applicable.

Table 2. Acibenzolar-*S*-methyl mediated prevention of the spread of *Ralstonia solanacearum* in basal and upper stem tissues of tomato cultivars BHN 466 and Equinox in greenhouse assays

Treatment ^z	Stem infection with <i>R. solanacearum</i> (%) ^y			
	BHN 466		Equinox	
	Basal stem	Upper stem	Basal stem	Upper stem
Actigard	34	4	48	10
Untreated	44	10	74	30
Chi-square (χ^2)	2.10 NS	6.40*	14.20**	12.50**

^y Percent values were means of two repeated experiments; values followed by * and ** indicate significant difference at $P = 0.05$ and $P = 0.01$, respectively, based on χ^2 test. NS indicates not significant.

^z Acibenzolar-*S*-methyl (Actigard 50 WG) was applied twice before transplanting and four times after transplanting at weekly intervals. Seedlings were transplanted into 10-cm pots 3 days after pathogen inoculation. Stem sections were processed for *R. solanacearum* colonization 4 days after transplanting (prior to wilt development).

Table 3. Effect of acibenzolar-*S*-methyl on bacterial wilt incidence and yield of tomato cultivars BHN 466, Neptune, and Equinox under field conditions (Fall 2002, Quincy, FL)

Treatment ^{w,x}		Disease incidence (%) ^y			Yield (t/ha) ^{y,z}		
		df	F	P	df	F	P
BHN 466 + Actigard			21 c			30.1 a	
BHN 466			46 b			22.6 c	
Neptune + Actigard			25 c			27.9 ab	
Neptune			54 b			24.7 bc	
Equinox + Actigard			100 a			0 d	
Equinox			100 a			0 d	
ANOVA sources		df	F	P	df	F	P
Actigard		1	15.04	<0.001	1	9.34	0.006
Cultivar		2	83.74	<0.001	2	228.70	<0.001

^w Acibenzolar-*S*-methyl (ASM; Actigard 50 WG) was applied twice before transplanting and six times after transplanting.

^x BHN 466 and Neptune are moderately resistant cultivars, and Equinox is susceptible to bacterial wilt.

^y Disease incidence was the final percentage of plants wilted. Yield and disease incidence values were means drawn from six replications. Same letter in each column indicates no significant difference according to Duncan's multiple range test at $P = 0.05$. Interactions between Actigard and cultivars are not significant, and only main effects are presented.

^z This represents a yield increase of 33.2% by ASM application for the cultivar BHN 466 and 13.0% for Neptune.

out of three experiments in moderately resistant cultivar BHN 466. In the 2003 field trial, it also significantly reduced bacterial wilt incidence in another moderately resistant cultivar, FL 7514. These results indicate that ASM increased host resistance to bacterial wilt of tomato from moderate to a significantly higher level and hence has the potential to be used for management of bacterial wilt on moderately resistant cultivars in tomato production.

ASM was not effective in reducing bacterial wilt incidence in the susceptible tomato cultivars when high concentrations of *R. solanacearum* were used in this study. This result was consistent with our previous findings that ASM did not reduce bacterial wilt incidence on a susceptible cultivar Solar Set under greenhouse conditions when inoculum concentrations higher than 10^7 CFU/ml were used (3). When relatively lower concentrations of *R. solanacearum* were used (10^5 or 10^6 CFU/ml), ASM provided consistent and significant protection against bacterial wilt in Solar Set (3). Under natural field conditions, soilborne *R. solanacearum* populations declined rapidly after the infected crop was harvested (26); hence, pathogen populations in some natural soils are not expected to be as high as that used in this study. Consequently, it deserves further studies to evaluate the efficacy of ASM in controlling bacterial wilt on susceptible tomato cultivars under natural field conditions where low inoculum concentrations exist.

Additional evidence for induced resistance following ASM application came from the differences in *R. solanacearum* populations in basal and upper stems of BHN 466 and Equinox, which are moderately resistant and susceptible to bacterial wilt, respectively. ASM treatment was associated with reduced upward movement of the pathogen in stem tissues irrespective of host genotype. This is consistent with previous reports that the bacterial wilt

resistance mechanism in tomato probably involved prevention of the spread of *R. solanacearum* in upper stem tissues (8,13). It is possible that multiplication of *R. solanacearum* in planta is prevented in xylem vessels in the basal stem. As a result, the movement of *R. solanacearum* toward upper parts of the stem was reduced. It is also noteworthy that initial colonization and migration of *R. solanacearum* in tomato plants appeared to be related to host resistance, as indicated by the numerically lower proportion of basal and upper stems harboring the pathogen in the moderately resistant cultivar BHN 466 compared with that of the susceptible cultivar Equinox. This finding was in agreement with McGarvey et al. (21), who reported that ingress of *R. solanacearum* into the taproot was faster and colonization by the pathogen in the midstems occurred sooner in a susceptible tomato cultivar than in resistant cultivars. Prevention of colonization and upward movement of the pathogen in stems mediated by ASM may be related to the reduction of disease incidence.

None of the ASM-treated asymptomatic plants was free from latent infection in the basal stem 30 days after transplanting in the greenhouse experiments (data not shown). Vascular tissues were brown in some of the systemically infected plants. Vascular browning might have been caused by invasion by *R. solanacearum*, rather than the application of ASM, since this discoloration was shown in both ASM-treated and untreated plants. The importance of latent infection in tomato plants by *R. solanacearum* on the reduction of tomato yield or dissemination of the bacterial pathogen is unclear. Further studies on these issues may be useful since latent infection can disseminate the bacterial pathogen in vegetatively propagated crops like potato, ginger, geranium, and banana (6).

ASM was shown to provide control of several diseases on tomatoes under field

conditions, including bacterial spot and bacterial speck (1,16,19,38), early blight and leaf mold (16), anthracnose (1), and spotted wilt caused by *Tomato spotted wilt virus* (23). Field experiments conducted in this study indicated that ASM reduced incidence of bacterial wilt on the moderately resistant tomato cultivars. ASM acts as a functional analog of salicylic acid in the SAR signaling pathway (18,24) and was reported to induce defense reactions in tomato plants such as the accumulation of pathogenesis-related proteins and ultrastructural changes (5,16). Negative impact of ASM on plant growth or yield has been shown in some cases (4,7,19,31,36). However, no yield reduction due to ASM applications was observed on noninoculated plants in multiple years' field studies in north Florida and south Georgia (S. M. Olson, unpublished data), nor were adverse effects of ASM observed for the rate used in this study in the greenhouse or field trials. An indication was that tomato yield of the moderately resistant cultivars was increased considerably by ASM application in both field trials of 2002 and 2003.

This is the first report of ASM-mediated control of bacterial wilt under field conditions on moderately resistant cultivars. In warm subtropical and tropical areas of the world, it is not economical to grow tomatoes in *R. solanacearum*-infested soils unless they are resistant to bacterial wilt. However, bacterial wilt resistant cultivars have not been generally accepted by the commercial tomato industry in the southeastern United States due to poor horticultural quality or insufficient disease resistance. For example, the resistant cultivars used in this study are only moderately resistant to bacterial wilt, and significant losses due to bacterial wilt continue to occur. It may be beneficial if the effect of ASM shown in this study can be used to enhance the level of resistance in moderately resistant cultivars; hence, these cultivars may gain more market acceptance. While this study showed the use of ASM to enhance resistance of some moderately resistant cultivars against *R. solanacearum*, further studies need to be conducted before practical use of ASM for management of bacterial wilt of tomato can be recommended to producers. For example, timing and dosage of ASM application should be optimized and standardized for achieving maximum efficacy and economic benefit in large-scale field experiments with more cultivars. The integrated effect of ASM in control of bacterial wilt as well as other tomato diseases can be evaluated under various field conditions. These studies will provide a more comprehensive evaluation of the impact of ASM in the production of tomatoes and may facilitate the feasibility for its practical application in control of bacterial wilt.

Table 4. Effect of acibenzolar-S-methyl on bacterial wilt incidence and yield of tomato cultivars FL 7514, Neptune, and FL 47 under field conditions (Fall 2003, Quincy, FL)

Treatment ^{w,x}	Disease incidence (%) ^y			Yield (t/ha) ^{y,z}		
FL 7514 + Actigard	18.6 c			41.7 a		
FL 7514	40.2 b			31.5 b		
Neptune + Actigard	10.8 c			44.0 a		
Neptune	30.4 b			39.3 ab		
FL 47 + Actigard	87.3 a			7.6 c		
FL 47	96.1 a			3.0 c		
ANOVA sources	df	F	P	df	F	P
Actigard	1	34.5	<0.001	1	7.06	0.01
Cultivar	2	248.5	<0.001	2	86.88	<0.001

^w Acibenzolar-S-methyl (ASM; Actigard 50 WG) was applied twice before transplanting and six times after transplanting.

^x FL 7514 and Neptune are moderately resistant cultivars, and FL 47 is susceptible to bacterial wilt.

^y Disease incidence was the final percentage of plants wilted. Values were means of six replications. Same letter in each column indicates no significant difference according to Duncan's multiple range test at $P = 0.05$. Interactions between Actigard and cultivars are not significant, and only main effects are presented.

^z This represents a yield increase of 32.2% by ASM application for the cultivar FL 7514 and 11.8% for Neptune.

ACKNOWLEDGMENTS

This research was sponsored in part by the Southern Region IPM (Jones, Momol, Olson, Grant # 2002-34103-11934) and T-STAR (Jones, Momol, Olson, 2002-3413512348) programs of USDA. We thank Jim Marois for help with regard to statistical analyses. We also acknowledge Hank Dankers, Anith Narayanan, Paula King, Sara Hague, and Bhuwan Pradhanang for technical assistance with processing tomato stem samples for latent infection detection. Contribution from the Florida Agricultural Experiment Station (Journal Series R-10085) is appreciated.

LITERATURE CITED

- Abbasi, P. A., Al-Dahmani, J., Sahin, F., Hoitink, H. A. J., and Miller, S. A. 2002. Effect of compost amendments on disease severity and yield of tomato in conventional and organic production systems. *Plant Dis.* 86:156-161.
- Akiew, E., and Trevorrow, P. R. 1994. Management of bacterial wilt of tobacco. Pages 179-198 in: *Bacterial Wilt: The Disease and Its Causative Agent, Pseudomonas solanacearum*. A. C. Hayward and G. L. Hartman, eds. CAB International, Wallingford, UK.
- Anith, K. N., Momol, M. T., Kloepper, J. W., Marois, J. J., Olson, S. M., and Jones, J. B. 2004. Efficacy of plant growth-promoting rhizobacteria, acibenzolar-S-methyl, and soil amendment for integrated management of bacterial wilt on tomato. *Plant Dis.* 88:669-673.
- Baldwin, I. T. 1998. Jasmonate-induced responses are costly but benefits plants under attack in native populations. *Proc. Natl. Acad. Sci. USA* 95:8113-8118.
- Benhamou, N., and Belanger, R. R. 1998. Benzothiadiazole-mediated induced resistance to *Fusarium oxysporum* f. sp. *radicislycopersici* in tomato. *Plant Physiol.* 118:1203-1212.
- Ciampi, L., Sequeira, L., and French, E. R. 1980. Latent infection of potato tubers by *Pseudomonas solanacearum*. *Am. Potato J.* 57:377-386.
- Csinos, A. S., Pappu, H. R., McPherson, R. M., and Stephenson, M. G. 2001. Management of *Tomato spotted wilt virus* in flue-cured tobacco with acibenzolar-S-methyl and imidacloprid. *Plant Dis.* 85:292-296.
- Elphinstone, J. G. 1994. Virulence of isolates of *Pseudomonas solanacearum* from worldwide sources on resistant and susceptible tomato cultivars. *Proc. Int. Conf. Plant Pathogenic Bacteria*, 8th. M. Lemattre, S. Freigoun, K. Rudolph, and J. G. Swings, eds. Versailles, France.
- Elphinstone, J. G., and Aley, P. 1993. Integrated control of bacterial wilt of potato in the warm tropics of Peru. Pages 277-283 in: *Bacterial Wilt*. G. L. Hartman and A. C. Hayward, eds. ACIAR Proc. No. 45. Canberra, Australia.
- Elphinstone, J. G., Hennessy, J., Wilson, J. K., and Stead, D. E. 1996. Sensitivity of different methods for the detection methods for the detection of *Ralstonia solanacearum* in potato tuber extracts. *EPPO/OEPP Bull.* 26:663-678.
- Engelbrecht, M. C. 1994. Modification of a semi-selective medium for the isolation and quantification of *Pseudomonas solanacearum*. A. C. Hayward, ed. ACIAR Bacterial Wilt Newsl. 10:3-5. Canberra, Australia.
- Gorlach, J., Volrath, S., Knaufbeiter, G., Hengy, G., Beckhove, U., Kogel, K. H., Oostendorp, M., Staub, T., Ward, E., Kessmann, H., and Ryals, J. 1996. Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. *Plant Cell* 8:629-643.
- Grimault, V., Anais, G., and Prior, P. 1994. Distribution of *Pseudomonas solanacearum* in the stem tissues of tomato plants with different levels of resistance to bacterial wilt. *Plant Pathol.* 43:663-668.
- Hanson, P. M., Wang, J.-F., Licardo, O., Hanudin, Mah, S. Y., Hartman, G. L., Lin, Y.-C., and Chen, J. 1996. Variable reaction of tomato lines to bacterial wilt evaluated at several locations in Southeast Asia. *HortScience* 31:143-146.
- Hayward, A. C. 1994. The hosts of *Pseudomonas solanacearum*. Pages 9-24 in: *Bacterial Wilt: The Disease and Its Causative Agent, Pseudomonas solanacearum*. A. C. Hayward and G. L. Hartman, eds. CAB International, Wallingford, UK.
- Inbar, M., Doostdar, H., Sonoda, R. M., Leibe, G. L., and Mayer, R. T. 1998. Elicitors of plant defensive systems reduce insect densities and disease incidence. *J. Chem. Ecol.* 24:135-149.
- Kelman, A. 1953. The bacterial wilt caused by *Pseudomonas solanacearum*. A literature review and bibliography. *N.C. Agric. Exp. Stn. Tech. Bull.* 99:1-194.
- Lawton, K. A., Friedrich, L., Hunt, M., Weymann, K., Delaney, T. P., Kessmann, H., Staub, T., and Ryals, J. 1996. Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. *Plant J.* 10:71-82.
- Louws, F. J., Wilson, M., Campbell, H. L., Cuppels, D. A., Jones, J. B., Shoemaker, P. B., Sahin, F., and Miller, S. A. 2001. Field control of bacterial spot and bacterial speck of tomato using a plant activator. *Plant Dis.* 85:481-488.
- Matheron, M. E., and Porchas, M. 2002. Suppression of Phytophthora root and crown rot on pepper plants treated with acibenzolar-S-methyl. *Plant Dis.* 86:292-297.
- McGarvey, J. A., Denny, T. P., and Schell, M. A. 1999. Spatial-temporal and quantitative analysis of growth and EPS I production by *Ralstonia solanacearum* in resistant and susceptible tomato cultivars. *Phytopathology* 89:1233-1239.
- Momol, M. T., Norelli, J. L., and Aldwinckle, H. S. 1999. Evaluation of biological control agents, systemic acquired resistance inducers and bactericides for the control of fire blight on apple blossom. *Acta Hort.* 489:553-557.
- Momol, M. T., Olson, S. M., Funderburk, J. E., Stavisky, J., and Marois, J. J. 2004. Integrated management of tomato spotted wilt on field-grown tomatoes. *Plant Dis.* 88:882-890.
- Oostendorp, M., Kunz, W., Dietrich, B., and Staub, T. 2001. Induced disease resistance in plants by chemicals. *Eur. J. Plant Pathol.* 107:19-28.
- Opena, T. T., Hartman, G. L., Chen, J. T., and Yang, C. H. 1990. Breeding for bacterial wilt resistance in tropical tomato. Pages 44-50 in: *Proc. Int. Conf. Plant Prot. Trop. 3rd. Genting Highlands, Pahang, Malaysia*.
- Pradhanang, P. M. 1998. Effects of crop rotation on bacterial wilt of potato and survival of race 3 of *Pseudomonas solanacearum* in the hills of Nepal. *Proc. Int. Conf. Plant Pathogenic Bacteria*, 9th. A. Mahadevan, ed. University of Madras, Chennai, India.
- Pradhanang, P. M., Elphinstone, J. G., and Fox, R. T. V. 2000. Identification of crop and weed hosts of *Ralstonia solanacearum* biovar 2 in the hills of Nepal. *Plant Pathol.* 49:403-413.
- Pradhanang, P. M., and Momol, M. T. 2001. Survival of *Ralstonia solanacearum* in soil under irrigated rice culture and aquatic weeds. *J. Phytopathol.* 149:707-711.
- 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 Dis.* 87:423-427.
- Prior, P., Beramis, M., Clairon, M., Quiquampoix, H., Robert, M., and Schmit, J. 1993. Contribution to integrated control against bacterial wilt in different pedoclimatic situations: Guadeloupe experience. Pages 294-304 in: *Bacterial Wilt*. G. L. Hartman and A. C. Hayward, eds. ACIAR Proc. No. 45. Canberra, Australia.
- Romero, A. M., Kousik, C. S., and Ritchie, D. F. 2001. Resistance to bacterial spot in bell pepper induced by acibenzolar-S-methyl. *Plant Dis.* 85:189-194.
- Scott, J. W., Jones, J. B., and Somodi, G. C. 1995. 'Neptune', a heat-tolerant, bacterial-wilt tolerant tomato. *HortScience* 30:641-642.
- Somodi, G. C., Jones, J. B., and Scott, J. W. 1993. Comparison of inoculation techniques for screening tomato genotypes for bacterial wilt resistance. Pages 120-123 in: *Bacterial Wilt*. G. L. Hartman and A. C. Hayward, eds. ACIAR Proc. No. 45. Canberra, Australia.
- Stavisky, J., Funderburk, J., Brodbeck, B. V., Olson, S. M., and Andersen, P. C. 2002. Population dynamics of *Frankliniella* spp. and tomato spotted wilt incidence as influenced by cultural management tactics in tomato. *J. Econ. Entomol.* 95:1216-1221.
- Sticher, L., Mauchmani, B., and Metraux, J. P. 1997. Systemic acquired resistance. *Annu. Rev. Phytopathol.* 35:235-270.
- Stout, M. J., Brovont, R. A., and Duffey, S. S. 1998. Effect of nitrogen availability on expression of constitutive and inducible chemical defenses in tomato, *Lycopersicon esculentum*. *J. Chem. Ecol.* 24:945-963.
- Wang, J.-F., Hanson, P., and Barnes, J. A. 1998. Worldwide evaluation of an international set of resistance sources to bacterial wilt in tomato. Pages 269-275 in: *Bacterial Wilt Disease: Molecular and Ecological Aspects*. P. Prior, C. Allen, and J. Elphinstone, eds. Springer Verlag, Berlin (DE).
- Wilson, M., Campbell, H. L., Ji, P., Jones, J. B., and Cuppels, D. A. 2002. Biological control of bacterial speck of tomato under field conditions at several locations in North America. *Phytopathology* 92:1284-1292.