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Management of bacterial wilt in tomatoes with thymol and acibenzolar-*S*-methyl

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ABSTRACT

The combination of thymol, a monoterpene phenol compound originating from thyme, and acibenzolar-*S*-methyl (ASM; Actigard 50 WG), a systemic acquired resistance (SAR) inducer, was applied to tomato plants in field conditions to evaluate the effectiveness of both chemicals to control bacterial wilt. Thymol was applied as a soil fumigant at 9.43 kg per ha 24 h after soil infestation and seven days before transplanting. ASM was applied as a foliar spray at 3.59–8.98 ml per ha, once in the greenhouse and five times in the field. The field was inoculated by applying 50 ml of pathogen suspension (10^7 cfu/ml) into each transplanting hole eight days prior to transplanting. The experiment was performed in 2006 and repeated in 2008 at the North Florida Research and Education Center in Quincy, FL. In 2006, the combination of ASM and thymol significantly reduced disease in the bacterial wilt tolerant genotype 7514 compared to thymol alone. In 2008, the combination of ASM and thymol significantly reduced disease and increased yield compared to the control, whereas ASM or thymol alone did not significantly reduce disease or increase yield compared to the control. This is the first report of the use of both thymol and ASM to control bacterial wilt on moderately resistant tomato cultivars. Based on this study, control of the pathogen can be achieved by using both chemicals and moderately resistant cultivars.

1. Introduction

Bacterial wilt, caused by the soilborne pathogen *Ralstonia solanacearum* (Smith) Yabuuchi, occurs worldwide in tropical and subtropical regions of the world (Yabuuchi et al., 1995). The bacterium can cause disease symptoms in over 200 different plant species (Buddenhagen and Kelman, 1964; Hayward, 1991). In the southeastern United States, economic losses for important solanaceous crops including tomato, tobacco and eggplant can be attributed to bacterial wilt. The bacterium enters the plant through the root and colonizes the vascular tissue in the stem. In field conditions, signs of the disease usually appear in mature tomato plants. Leaves will wilt during the day and recover at night or during the early hours of the morning. If the weather is favorable, with high humidity and high temperatures, the disease can cause complete wilting of the plant and eventually death. In the advanced stages of wilt, the leaves of wilted plants remain green and the vascular tissue usually turns a brownish yellow. In the field, the disease occurs mostly in areas where water accumulates; however, plants showing signs of the disease can be found sporadically throughout. Plants affected by *R. solanacearum* can also be stunted, due to the lack of water and poor uptake of nutrients.

Current integrated management strategies include the use of resistant cultivars, pathogen-free transplants and crop rotation with non-host cover crops (Pradhanang et al., 2005). However, these strategies have proven to be limited due to the complex nature of soilborne pathogens. Resistant cultivars have been developed for fresh market production in the U.S.; however, the growers have only adopted moderately resistant cultivars (Scott et al., 1995). Resistant and moderately resistant cultivars are limited in terms of location, climate and resistance to strains of the pathogen (Saddler, 2004). Transplants limit the spread of the bacterium, yet due to it being a soilborne pathogen, most plants in the field can be infected. Cover crops or crop rotation can be difficult due to the diverse host range of *R. solanacearum* strains, and the fact that the pathogen is able to survive or colonize various weeds that surround the field (Hayward, 1991). With the limited control measures and the gravity of bacterial wilt on important economical crops, investigating other methods for controlling the disease has become critical.

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Plants are able to activate a protective mechanism after contact by a pathogen, these plant metabolites, or by a diverse group of structurally unrelated organic and inorganic compounds. This phenomenon has been dubbed as systemic acquired resistance (SAR) (Kuc, 2001). SAR inducers are ideal for controlling diseases because they trigger a response that may protect the plant from fungal, bacterial and viral pathogens, if the product is applied at the correct time. Acibenzolar-*S*-methyl (ASM; Actigard 50 WG, Syngenta, Basel, Switzerland) is a chemical compound that triggers SAR when applied to plants (Oostendrop et al., 2001). ASM has been used to reduce the incidence of fire blight in pear and apple, bacterial spot and speck in tomato and pepper, and common bunt in wheat seedlings (Louws et al., 2001; Norelli et al., 2003; Obradovic et al., 2005; Lu et al., 2006). Previously it was reported that ASM enhanced host resistance in moderately resistant tomato cultivars against bacterial wilt (Pradhanang et al., 2005).

Thymol (2-isopropyl-5-methylphenol) is a monoterpene phenol derivative of thyme (Aeschbach et al., 1994). Essential oils have been used in the past for flavoring and preserving food, for their antioxidant power and for their antimicrobial activity (Scheie, 1989; Lambert et al., 2001; Rojano et al., 2008). Both medical and food sciences have shown that thymol is able to inhibit both Gram-positive and Gram-negative bacteria (Evans and Martin, 2000; Lambert et al., 2001; Walsh et al., 2003; Cailet and Lacroix, 2006; Shapira and Mimran, 2007). Previously thymol applied as a biofumigant was reported to effectively control bacterial wilt. Thymol applications in the field on susceptible tomato cultivars were able to reduce the incidence of bacterial wilt and increase yield (Ji et al., 2005).

In previous studies bacterial wilt was reduced by applying ASM in combination with moderately resistant tomato cultivars (Pradhanang et al., 2005), or by applying thymol and using susceptible tomato plants (Ji et al., 2005). In this study, we wanted to determine if using a combination of thymol, ASM and moderately resistant plants would elevate the level of efficacy in controlling bacterial wilt. This would be the first time that the two products had been applied together on moderately resistant tomato cultivars in a field trial. It was unknown if the chemicals would work synergistically or would have little to no effect in enhancing disease control. Success with both of the chemicals in controlling the disease would provide another tool in a small arsenal to control bacterial wilt.

2. Materials and methods

2.1. Bacterial culture and inoculum preparation

Ralstonia solanacearum strain RS5 isolated from tomato in Quincy, Florida, was used in this study (Pradhanang and Momol, 2001). Pathogenicity was determined by performing Koch's postulates by inoculating tomato plants and re-isolating RS5. Bacteria were plated on modified semi-selective agar, SMSA (Englebrecht, 1994), and casamino acid peptone glucose agar, CPG (Schaad et al., 2001). Plates were stored at 28 °C. The inoculum contained bacteria grown on CPG for 24 h and suspended in sterile deionized water. The bacterial suspension was adjusted to 10^7 CFU/ml using sterile deionized water. Inoculum concentration was estimated using a spectrophotometer (Sigma-Aldrich Co., Milwaukee, WI) at 600 nm. The actual bacterial concentration (cfu/ml) was determined by performing 10-fold dilutions of the inoculum suspension and plating on CPG. Where each tomato plant was to be transplanted, 15 cm holes were created in the soil and 50 ml of the bacterial suspension was poured into each hole (Ji et al., 2005). The field was infested 10 day prior to transplantation, in which time the field was treated with thymol and plants were treated with 1 application of ASM. The holes were covered with tape prior to the thymol fumigation.

2.2. Application of thymol and ASM

Thymol was applied as a soil fumigant 24 h after the field was infested. The field was aerated 7 days post thymol application, and transplanting occurred 3 days after field aeration. Thymol was applied at 9.42 kg per ha, in a solution consisting of water, 70% ethanol and detergent. ASM was applied as a foliar spray at a volume of 10 ml of ASM solution (25 μg/ml) per plant. The ASM solution was applied 6 times: 1 week before the seedlings were transplanted, 1 day after transplanting, followed by 2 treatments that were applied once a week and then 2 treatments that were applied biweekly.

2.3. Tomato plants and experimental design

In the 2006 trial, tomato cultivars 'Phoenix', 'FL7514' and 'BHN669' were used in the field experiment, the first being susceptible and the last two moderately resistant to bacterial wilt. For the 2008 trial, only 'Phoenix' and 'FL7514' were used. Tomato plants were grown in Terra-Lite agricultural mix (Scott Sierra Horticultural Products Co., Marysville, OH) in expanded polystyrene flats with 3.5 × 3.5 cm cells. For each experiment, 5-week-old tomato seedlings were transplanted 1 week after the thymol application.

The experiments for both years were conducted in experimental fields at the University of Florida North Florida Research and Education Center located in Quincy. Previously, the fields were used for growing tomatoes. The beds were fumigated with methyl bromide (67%) and chloropicrin (33%) at a broadcast equivalent rate of 392 kg a.i./ha for control of weeds and other soilborne pathogens, fertilized with 218-31-181 kg/ha of N–P–K and covered with polyethylene mulch 1 week prior to infestation of the field. The plots consisted of four rows, 5 m long with the raised beds, 10 cm high by 91 cm wide and centered 1.8 m apart. Tomato plants were treated with standard foliar sprays for insecticides and fungicides at weekly intervals until harvest. Over time the plants were tied and staked. A randomized complete block design was used including 6 blocks for each cultivar and treatment in 2006 and 4 blocks in 2008. Each block constituted a replication. Each block was 10–12 m long with 14 tomato seedlings transplanted per block in 2006 and 18 in 2008. Thus each treatment consisted of 84 plants per cultivar in 2006 and 72 plants per cultivar in 2008. In the 2006 experiment, each block of plants received one of the following treatments: thymol, the combination of thymol and ASM or neither thymol nor ASM, which was the untreated control (UTC). The treatments for the 2008 experiment consisted of thymol, ASM, both thymol and ASM or the UTC. In between each block was a 2 m buffer where no tomato seedlings were planted.

2.4. Disease and yield assessment and statistical analysis

Completely wilted tomato plants were removed from the field weekly and a few of the plants were tested for the presence of the bacterium. The confirmation of *R. solanacearum* was performed by a bacterial ooze test and either isolation on SMSA and confirmation by gas chromatographic profiling of whole-cell fatty acid methyl esters (FAME) (MIDI, Newark, DE), as described previously (Stead, 1992; Pradhanang et al., 2003), or by using *R. solanacearum* specific immunoassay strips (Agdia, Inc., Elkhart, IN). RS5 was used as positive control for each test. In both 2006 and 2008 completely wilted plants were counted weekly after transplanting. Bacterial wilt incidence was recorded at weekly intervals and was quantified