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# Application of acibenzolar-*S*-methyl and standard fungicides for control of Phytophthora blight on squash

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ABSTRACT

A plant systemic acquired resistance inducer, acibenzolar-*S*-methyl (ASM), was evaluated to determine the efficacy for suppression of Phytophthora blight of squash caused by *Phytophthora capsici* under field conditions. ASM was applied as foliar sprays before and after transplanting at rates of 17.5, 8.8, and 4.4 g a.i. $ha^{-1}$. Application of ASM did not significantly reduce final Phytophthora blight incidences in 2 out of 3 field experiments; however, area under the disease progress curve (AUDPC) values were reduced significantly by ASM in all experiments conducted. Disease suppression by the three application rates of ASM was not significantly different. To determine the effect of application of ASM in conjunction with standard chemical fungicides, ASM was applied with mefenoxam (Ridomil Gold), copper hydroxide, and mandipropamid (Revus). AUDPC values and final disease incidences were consistently lower in field plots treated by the combination of ASM and standard fungicides than applications of these chemicals alone. Application of ASM resulted in significantly higher squash yield than the non-treated control in 2 of 3 experiments and plots treated with the combined use of ASM and standard fungicides produced the highest yields. These results suggest that ASM may induce plant resistance under field conditions, providing suppression of Phytophthora blight of squash, and that there may be some benefit to the integration of ASM and standard chemical fungicides.



## 1. Introduction

Phytophthora blight is a serious disease on cucurbits, peppers, eggplants, and a number of other important vegetable crops in the United States and worldwide (Erwin and Ribeiro, 1996; Hausbeck and Lamour, 2004; Ristaino and Johnston, 1999; Sanogo and Carpenter, 2006; Sholberg et al., 2007). The disease is incited by the soilborne pathogen *Phytophthora capsici* that causes root rot, crown rot, seedling damping-off, leaf and stem blight, fruit rot, and plant wilt. The destructive nature of the disease and the wide host range and geographical distribution of the pathogen has made Phytophthora blight one of the most important diseases on vegetables.

Squash (*Cucurbita pepo*) is among the most susceptible crops to Phytophthora blight and management of the disease on squash is difficult. Commercial cultivars of squash resistant to *P. capsici* are not yet available. Crop rotation with non-host crops is a commonly practiced strategy for control of soilborne pathogens (Cook, 1991), however, cropping sequences that can be used to efficiently manage Phytophthora blight have not been established. *P. capsici* has a broad crop and weed host range and produces thick-walled oospores that survive in soils for extended period of time (Erwin and Ribeiro, 1996; Ploetz et al., 2002; Tian and Babadoost, 2004), which may reduce the efficacy of crop rotation. At present, application of selected fungicides continues to be an important component in developing integrated programs for managing Phytophthora blight. Traditionally, fungicides containing active ingredient mefenoxam have been used for control of the disease. However, disease control is hampered by the presence of mefenoxam-resistant strains that have developed in Georgia and other vegetable production areas (Café-Filho and Ristaino, 2008; Gevens et al., 2007; Lamour and Hausbeck, 2000; Mathis, 1999; Wang et al., 2009). In recent years some newer fungicides such as mandipropamid have become available for control of diseases caused by *Phytophthora* spp. (Jang et al., 2009). Although no isolates of *P. capsici* in Georgia have been found to be resistant to mandipropamid so far (Ji et al., unpublished), due to the remarkable ability of the pathogen to develop resistance to fungicides, development of alternative or complementary fungicides with different mode of actions is highly desirable.

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The plant can activate protective mechanisms following contact by a pathogen or invader, which is termed systemic acquired resistance (Sticher et al., 1997). Systemic acquired resistance (SAR) can be induced by microorganisms, metabolic substances of the host plant, or chemical compounds (Achuo et al., 2004; Kessmann et al., 1994; Métraux et al., 1990). A SAR inducer that has been extensively studied for control of plant diseases in recent years is acibenzolar-*S*-methyl (ASM). ASM acts as a functional analog of salicylic acid in the SAR signaling pathway and enhances expression of resistance related genes and activities of various enzymes, lignin, and phenolic compounds (Benhamou and Nicole, 1999; Bokshi et al., 2003; Buzi et al., 2004; Malolepsza, 2006; Soylu et al., 2003). Application of ASM suppressed Phytophthora blight on pepper caused by *P. capsici* under greenhouse conditions (Matheron and Porchas, 2002) and crown rot and red stele root rot on strawberry caused by *Phytophthora cactorum* and *P. fragariae* var. *fragariae* (Eikemo et al., 2003). It was also demonstrated that ASM reduced other diseases caused by Oomycetes (LaMondia, 2008; Leskovar and Kolenda, 2002; Tosi et al., 1999), fungi (Buzi et al., 2004; Elmer, 2006; Willingham et al., 2002), bacteria (Ji et al., 2007; Louws et al., 2001; Soylu et al., 2003; Wilson et al., 2002) and viruses (Csinos et al., 2001). In our previous studies, ASM provided significant suppression of Phytophthora blight on squash under greenhouse conditions (Koné et al., 2009). It is evident that ASM has broad-spectrum activities in control of various plant diseases, however, the capability of ASM in management of Phytophthora blight on squash under field conditions has not been documented.

The objective of this study was to evaluate the efficacy of ASM in controlling Phytophthora blight of squash under field conditions. In addition, integration of ASM with traditional fungicides used for control of this disease was also evaluated to determine the potential for further improving disease suppression.

## 2. Materials and methods

### 2.1. Chemical fungicides used in the study

The products used in the study, including acibenzolar-*S*-methyl (ASM, Actigard 50WG), Ridomil Gold (a.i. mefenoxam), Ridomil Gold Copper (a.i. mefenoxam and copper hydroxide), and Revus (a.i. mandipropamid), were obtained from Syngenta Crop Protection (Greensboro, North Carolina, USA).

### 2.2. Determination of efficacy of acibenzolar-S-methyl

Squash seeds (cv. Dixie) were sown in expanded polystyrene flats with 3.5 × 3.5 cm cells containing a potting mix (Miracle-Gro Lawn Products, Inc., Marysville, OH, USA) and incubated in a greenhouse. ASM (Actigard 50WP) was applied by foliar spray two weeks after squash seedling emergence at concentrations of 36, 18, and 9 μg ml$^{-1}$. For foliar spray, a volume of 100 ml of ASM solution was applied onto the foliage of 20 plants using a hand held garden sprayer. The plants were maintained in the greenhouse with temperatures of 22–26 °C (night) and 28–35 °C (day).

A field experiment was conducted at University of Georgia Coastal Plain Experiment Station in Tifton, Georgia in summer 2007. The soil type was fuquay loamy sand (88% sand, 8% silt, 4% clay; pH 5.5–6.0; 2% organic matter). The field site has a history of *P. capsici* with moderate infestation of the pathogen in the soil. Raised beds were prepared for plant growth, 15-cm high by 75-cm-wide and centered 1.8 m apart. Beds were fertilized with 560 kg/ha of 10-10-10 (N-P-K) and covered with polyethylene mulch. A drip irrigation line (Aqua-Traxxs Premium drip tape, Toro Ag Irrigation Business, El Cajon, California, USA) was installed at a depth of 2.5 cm with a 30-cm emitter spacing and a flow rate of 5.6 L min$^{-1}$ per 100 m. The plots were irrigated to field capacity by drip irrigation prior to squash transplanting. Squash seedlings were planted 30 cm apart in a row on raised plastic-mulched beds 5 days after application of ASM in the greenhouse. Each individual experimental plot consisted of a single row which was 6 m long and buffer zones with 1.5 m spacing without planting of squash seedlings were maintained at the ends of each plot within the row. Each treatment was replicated four times in a randomized complete block design with 20 plants in each replication.

In the field, Actigard (a.i. ASM) was applied by foliar sprays at rates of 17.5, 8.8 and 4.4 g a.i. ha$^{-1}$, onto those plants treated with ASM in the greenhouse at 36, 18, and 9 μg ml$^{-1}$ respectively. Application of ASM in the field was made 2, 4, and 6 weeks after transplanting. Non-treated plots and the combination of Ridomil Gold (a.i. mefenoxam), Ridomil Gold Copper (a.i. mefenoxam and copper hydroxide) and Revus (a.i. mandipropamid) were used as controls. Ridomil Gold was used for soil treatment through drip irrigation at a rate of 1.5 L ha$^{-1}$at transplanting. Ridomil Gold Copper was applied by foliar sprays at a rate of 2.2 kg ha$^{-1}$1, 3, 5 weeks after transplanting. Revus was applied as foliar sprays at 0.6 L ha$^{-1}$ along with a wetting agent Activator (0.125% v/v) 2, 4, 6 weeks after transplanting. Foliar applications were made with a $CO_2$-powered sprayer calibrated to deliver approximately 470 L/ha. Insects were controlled using standard insecticides recommended by University of Georgia Cooperative Extension (Riley and Sparks, Vegetable Entomologists).

Symptomatic squash plants were sampled for isolation of *P. capsici* on PARP selective medium (Jeffers and Martin, 1986) containing the following ingredients in each liter of V8 juice agar medium: pimaricin, 5 mg; ampicillin, 250 mg; rifampicin, 10 mg; PCNB, 50 mg. Presumptive *P. capsici* isolates were purified using the methods of Fyfe and Shaw (1992) by subculturing hyphal tip on PARP medium and pathogen identity was verified by morphological characteristics (Waterhouse, 1963) and polymerase chain reaction (PCR) analysis using *P. capsici*-specific primers (Silvar et al., 2005). Plants with Phytophthora blight were counted weekly after first appearance of symptoms in the field and disease incidence was calculated as percentage of diseased plants. Area under the disease progress curve (AUDPC) was calculated using the formula described by Shaner and Finney (1977): $\sum_{i=1}^{n}[(Y_{i+n1}+Y_i)/2][X_{i+1}-X_i]$, where $Y_i$ = disease incidence at the $i$th observation, $X_i$ = time (days) at the $i$th observation, $n$ = total number of observation. AUDPC values for each treatment were calculated from six disease ratings 3–8 weeks after transplanting. Squash fruit were hand harvested when mature and marketable and unmarketable yields were determined. Disease and yield data were analyzed using the Statistical Analysis System (SAS Institute, Cary, NC, USA) and analysis of variance and Fisher's protected least significant difference (LSD) test were used to determine differences among treatments at $P = 0.05$.

### 2.3. Application of ASM in conjunction with conventional fungicides

Experiments were conducted in fall 2007 and summer 2009 to evaluate the efficacy of ASM further and determine the effect of application of ASM in conjunction with conventional fungicides. The experiments were conducted at University of Georgia Coastal Plain Experiment Station in Tifton, GA. Growth of squash seedlings (cv. Dixie), field preparation, and ASM application were as in the field experiment described above. A total of three foliar applications of ASM was made in the field 2, 3, 6 weeks after transplanting at rates of 17.5, 8.8, and 4.4 g a.i. ha$^{-1}$. For combined use of ASM and conventional fungicides, Ridomil Gold was applied at transplanting through drip irrigation at a rate of 1.5 L ha$^{-1}$. Revus was applied as