CARIBBEAN FOOD CROPS SOCIETY 44

Forty Fourth
Annual Meeting 2008

Miami, Florida, USA

Vol. XLIV – Number 2
Plenary Session and Oral Presentations

MEETING HOST:

UF UNIVERSITY of FLORIDA IFAS

2008 Proceedings of the Caribbean Food Crops Society. 44(2):212-216. 2008

Acibenzolar-S-Methyl and PGPR Increases Host Resistance in Squash to Phytophthora Blight Under Greenhouse Conditions

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ABSTRACT.

Phytophthora blight disease caused by *Phytophthora capsici* Leonian threatens vegetable production worldwide. It is imperative to develop practical and cost-effective alternatives to methyl bromide in order to sustain production of vulnerable vegetables including squash in Florida. Greenhouse studies have been conducted to evaluate the potential of the use of Acibenzolar-S-methyl (ASM; Actigard 50WG) and plant growth-promoting rhizobacteria (PGPR; SE34 and IN973b) for control of Phytophthora blight on squash. Actigard at 30 and 3 mg/l and PGPR strain SE34 at 10^7 CFU/ml significantly ($P<0.05$) reduced disease severity of Phytophthora blight of squash compared to the nontreated control. Actigard at 30 mg/l provided the greatest protection in squash against *P. capsici*. No disease symptom developed on squash plants treated with Actigard at 30 mg/l while nontreated inoculated plants collapsed due to the infection of *P. capsici*. The results suggest that ASM and PGPR are effective against *P. capsici* in squash and may be incorporated in integrated management strategies for control of Phytophthora blight of squash.

KEYWORDS: *Phytophthora capsici*, induced disease resistance, squash

INTRODUCTION

Phytophthora blight, caused by the oomycete *Phytophthora capsici*, is one of the most serious threats to cucurbit production in the United States and worldwide (Babadoost 2004; Hausbek and Lamour 2004). *P. capsici* is a soil-borne pathogen and survives as oospores for many years in soil or as mycelia in plant debris. *P. capsici* infects a wide range of plant taxa involving a total of more than 50 species (Tian and Babadoost 2004), including major vegetable crops and weeds. Recently, the incidence of Phytophthora blight has dramatically increased in many cucurbit-growing areas, causing up to 100% yield loss (Babadoost 2004; Hausbek and Lamour 2004). Summer squash in south Florida is highly susceptible to *P. capsici* causing foliar blight and fruit rot (Roberts *et al.* 2001), and winter squash is also of concern to Florida producers.

P. capsici can infect squash plants at any stage of growth. Symptoms of Phytophthora blight include seed rot, seedling damping-off, leaf and vine blight, and fruit rot. Considering the pathogen's tremendous ability to reproduce, and given the optimal conditions of warm, wet weather in south Florida, *P. capsici* can devastate a whole field of squash within several days. In 2007, several squash growers in Miami-Dade County, Florida, who produced calabaza for the Florida and New Jersey markets for many years,

have ceased this line of operation because of disastrous crop losses from Phytophthora blight (Julian Guillarte, personal communication).

Practices available for management of soil-borne pathogens such as *P. capsici* in the field include cultural practices, crop rotation, fungicide applications, pre-plant fumigation and the use of resistant or tolerant varieties. At present, there is no single method which can provide adequate control of *P. capsici* (Babadoost 2004; Hausbek and Lamour 2004). Highly resistant varieties with ideal horticultural traits are not available for Florida (Olson *et al.* 2007). Crop rotation is an important component of integrated disease management; however, the long-term survival of *P. capsici* oospores even in the absence of a host limits the effectiveness of this strategy (Hausbek and Lamour 2004). There is a limited number of fungicides registered for use on cucurbits and no fungicides are highly effective against *P. capsici* (Babadoost 2004; Hausbek and Lamour 2004). More importantly, *P. capsici* has been reported to develop resistance to some fungicides applied for Phytophthora blight control (Hausbek and Lamour 2004).

Methyl bromide, a fumigant used extensively to control soil-borne pathogens, is effective against the mycelia and the long-term persistent oospores of *P. capsici* in the soil. However, agricultural emissions of methyl bromide have been shown to be a significant source of ozone depletion (EPA 2008; Spreen *et al.* 1995). Therefore, the phase-out of the use of methyl bromide has been ongoing under the 1989 Montreal Protocol. Consequently, many tomato and pepper growers in Florida are replacing the use of a mixture of methyl bromide and chloropicrin with a combination of 1, 3-dichloropropene (a nematicide) and herbicides; however, 1, 3-dichloropropene cannot be used in counties with karst geography, such as Miami-Dade County. Metam sodium and chloropicrin have been registered for control of *P. capsici* (Hausbek and Lamour 2004), but are less reliable than methyl bromide and chloropicrin mixtures. Methyl iodide and chloropicrin mixtures are highly effective against *P. capsici* and have been tested extensively for protection of tomato (Rosskopf *et al.* 2005). Recently, a 50:50 mixture of methyl iodide (MIDAS®) has been registered in Florida for use on ornamentals, strawberries, tomatoes, peppers, tree fruit, nuts and vines, as well as turf. (Arysta LifeScience Corp., 2008). However, the high cost of methyl iodide is likely to be a prohibitive factor for use of this product in squash production. Spreen *et al.* (1995) estimated that the loss of methyl bromide would result in a $1 billion impact on the US winter vegetable industry, mostly borne by Florida producers. The objective of this study was to evaluate acibenzolar-S-methyl (ASM) and plant growth-promoting rhizobacteria (PGPR) for their effect on Phytophthora blight of squash in the greenhouse.

MATERIALS AND METHODS

Greenhouse experiments were conducted with squash F1 hybrid HMX 5703 (provided by R. See, Seedway-SE Florida). Seeds of squash were planted in transplant flats containing potting mix. Two applications of the inducing agents were performed by soil drench at 1 and 2 weeks after planting (WAP), respectively. The treatments were ASM (Actigard® 50 WG, Syngenta, Inc.) at 30 and 3 mg/l, PGPR strains SE 34 and IN937b each at 1×10^7 CFU/ml, and silicic acid at 1.5 and 0.5 mM. Nontreated plants served as the blank control. Five milliliters of the cocktail inoculum (2×10^4 spores/ml), which contains equal number of zoospores for each of the three isolates (#121, #146 and #151, provided by Dr. P. Roberts) were applied to each plant at the time of inoculation by

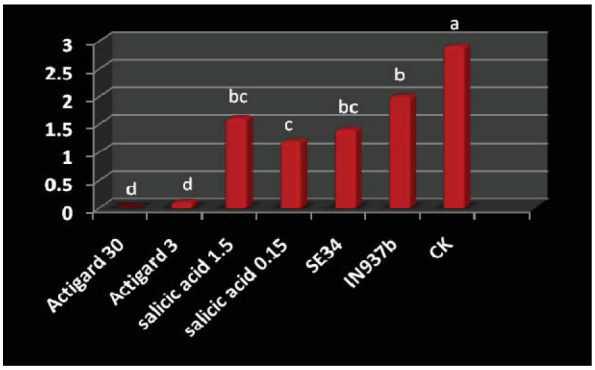
adding to the soil around the stem. Inoculated plants were placed on benches for 7 days when disease was rated according to a disease rating scale of 0-5. In this rating scale, 0 = no visible symptoms, 1 = small brownish lesion at the base of stem, 2 = stem lesions extend to cotyledons or the lesion has girdled the stem causing plant collapse, 3 = plants have collapsed with all leaves wilted or turned yellow except for the young leaves, 4 = plants have completely collapsed, and 5 = plants are dead. Treatments were arranged as randomized complete blocks with five replications and two plants per replication.

In other greenhouse assays, Actigard® was evaluated for the effect on Phytophthora blight of squash at various concentrations of 30, 20, 10, 1 and 0.1 mg/l. In an experiment to evaluate the potential of improved effect by combining Actigard® and PGPR strain SE34, Actigard® was applied at 10 and 3 mg/l alone and in combination with SE34. Actigard® at 30 mg/l served as the positive control, and nontreated squash plants served as the blank control. The experimental design and application method were the same as described above.

Data from greenhouse and field experiments were analyzed by analysis of variance using JMP software (SAS Institute Inc., Cary, NC). The significance of effects of treatments was determined by the magnitude of the F value (P = 0.05). When a significant F test was obtained for treatments, the separation of means was accomplished by Fisher's protected Least Significant Difference (LSD).

RESULTS AND DISCUSSION

In the repeated greenhouse assays, Actigard® at 30 and 3 mg/l and PGPR strain SE34 at 10^7 CFU/ml significantly ($P<0.05$) reduced disease severity of Phytophthora blight of squash compared to the nontreated control (Figure 1). ASM at 30 mg/l provided the greatest protection in squash against *P. capsici*. No disease symptoms developed on squash plants treated with SAM at 30 mg/l whereas nontreated inoculated plants collapsed due to the infection of *P. capsici*.



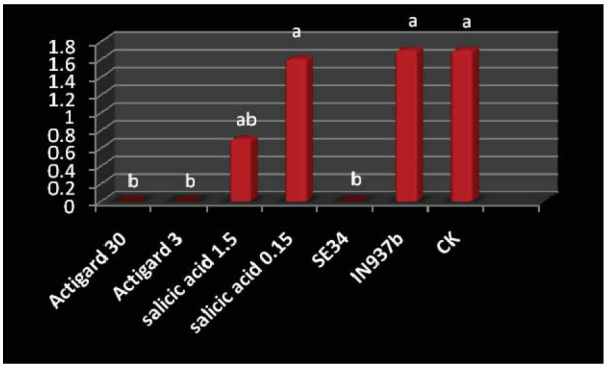


Figure 1. Effect of ASM and PGPR treatments on Phytophthora blight of squash in the greenhouse. Data presented are from two repeated greenhouse experiments. Values of disease severity with the sameletter are not statistically different (P=0.05, LSD).

Squash plants treated with Actigard® at 10 mg/l or higher concentrations consistently had less disease severity when compared with the nontreated plants (Figure 2). Treatments with Actigard® at 3 mg/l and 10 mg/l in combination with SE34 significantly reduced disease severity of *P. capsici* compared to the nontreated control, as well as to the treatment with SE34 alone (Figure 3). This indicates that improved control of Phytophthora blight in squash can be achieved in the greenhouse by combining ASM and PGPR strain, and field trials should be conducted to confirm this effect under field conditions.

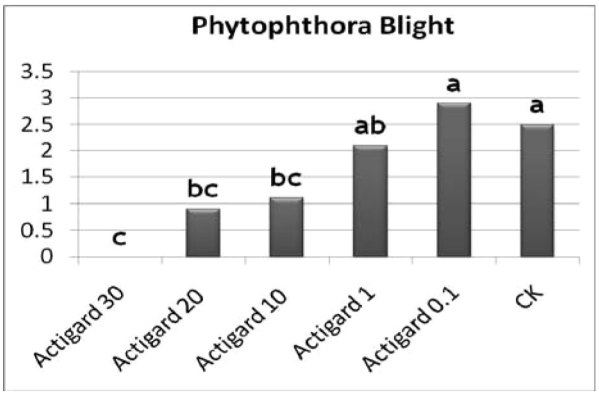


Figure 2. Effect of ASM on Phytophthora blight of squash in the greenhouse. Results from the repeated experiments were same. This is a representative from three repeated experiments. Values of disease severity with the same letter are not statistically different (P=0.05, LSD).

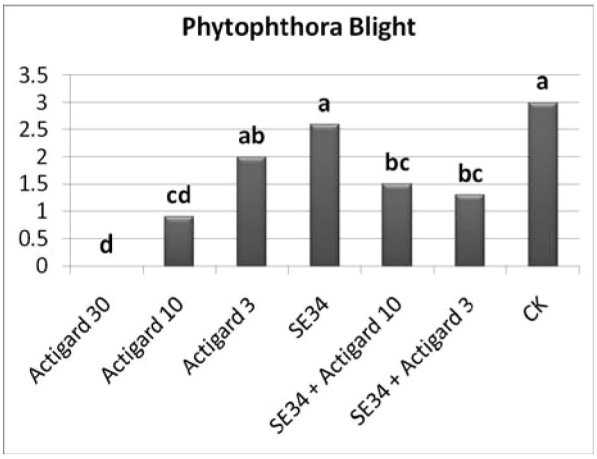


Figure 3. Combined effect of ASM and PGPR strain SE34 on Phytophthora blight of squash in the greenhouse. Results from the repeated experiments were similar. This is a representative from repeated experiments. Values of disease severity with the same letter are not statistically different (P=0.05, LSD).

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