Determining sensitivity of watermelon Fusarium wilt pathogen to chemical fungicides

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Introduction

Fusarium wilt of watermelon, caused by the fungal pathogen *Fusarium oxysporum* f. sp. *niveum* (FON), is responsible for significant yield loss in watermelon production. The disease is well established in nearly all watermelon growing regions in the United States and the world, and has been reported in Africa, Asia, Australia, Europe, North America, and South America (1). The pathogen may cause pre- or post-emergence damping-off of young seedlings and greenhouse transplants, and death of seedlings is rapid under favorable conditions (1). In the field, symptoms typically occur within 3 to 4 weeks, starting with a graying of foliage followed by foliar chlorosis and wilt. Plants affected late in the season may wilt and collapse or be stunted. Fusarium wilt is characterized by stem necrosis, which is easily visualized when runners or stems are sectioned (1, Figures 1 and 2).

Fig. 1: Fusarium wilt of watermelon in a commercial field in Georgia in 2013.

Fig. 2: Stem necrosis caused by FON.

Application of chemical fungicides continues to be a significant component in developing effective disease management programs. A number of fungicides have been demonstrated to be effective in reduction of the disease, including Quadris (a.i. azoxystrobin), Proline (a.i.

prothioconazole), and Topsin (a.i. thiophanate-methyl) (2). These fungicides have different mode of action, but they are all targeting single site. Development of resistance to fungicides with single site mode of action is common in populations of fungal pathogens, and isolates of FON may develop resistance to the fungicides. However, current status of resistance in FON populations prevalent in watermelon fields to the above mentioned fungicides is largely unknown. In addition, relative effectiveness of the fungicides in suppression of mycelial growth and sporulation of FON has not been documented. This study was conducted to determine the effect of prothioconazole, azoxystrobin, and thiophanate-methyl fungicides on *Fusarium oxysporum* f. sp. *niveum* and monitor fungicide resistance development in the pathogen populations in Georgia and Florida.

Materials and Methods

FON isolates: A collection of 120 isolates were obtained from watermelon fields at different locations in Georgia and Florida. Pathogenicity of the isolates was verified by inoculation of a susceptible watermelon cultivar under greenhouse conditions. The 120 isolates were used to determine development of resistance to prothioconazole, azoxystrobin, and thiophanate-methyl.

Percentage of resistant isolates: An agar plug (7 mm in diameter) taken from the edge of an actively growing colony was placed at the center of potato dextrose agar (PDA) plate amended with prothioconazole, azoxystrobin, or thiophanate-methyl at a final concentration of 100 mg/liter (ppm). For each fungicide, triplicate plates were used for each isolate and the plates were incubated at 25°C. Colony diameter was measured in two perpendicular directions 5 days after incubation and averaged for analysis. The relative growth rate of FON on fungicide amended and non-amended control plates was used to determine resistance to the fungicides (sensitive: <30% of the control, i.e. colony diameter on fungicide amended plates was less than 30% of colony diameter on non-amended control plates; intermediate sensitive: 30 to 90% of the control; resistance: >90% of the control). Percentage of resistant and sensitive isolates was calculated.

In addition, the effectiveness of the fungicides for suppression of spore production was determined. Twenty five isolates were grown as mentioned above and number of conidia produced per unit area was counted. Production of conidia on fungicide-amended and non-amended control plates was compared and analyzed.

Sensitivity to prothioconazole and azoxystrobin: Twenty five isolates were used to determine sensitivity to prothioconazole and azoxystrobin since no isolates were found to be resistant to the two fungicides. PDA plates were amended with the fungicides separately at different concentrations ranging from 0 to 10 mg/liter. An agar plug taken from the edge of an actively growing colony was placed at the center of the plates and growth of the fungus was measured as described above. Fungicide concentrations that reduce 50% of the growth of the fungus (EC_{50}) were calculated.

Results

Percentage of resistant isolates: When determined based on mycelial growth, 4% of the isolates were resistant to thiophanate-methyl, and 22.5% and 73.5% were sensitive and intermediately sensitive, respectively. All the isolates were sensitive to prothioconazole. No isolate was

resistant to azoxystrobin, and 24% and 76% were sensitive and intermediately sensitive to the compound, respectively (Figure 3).

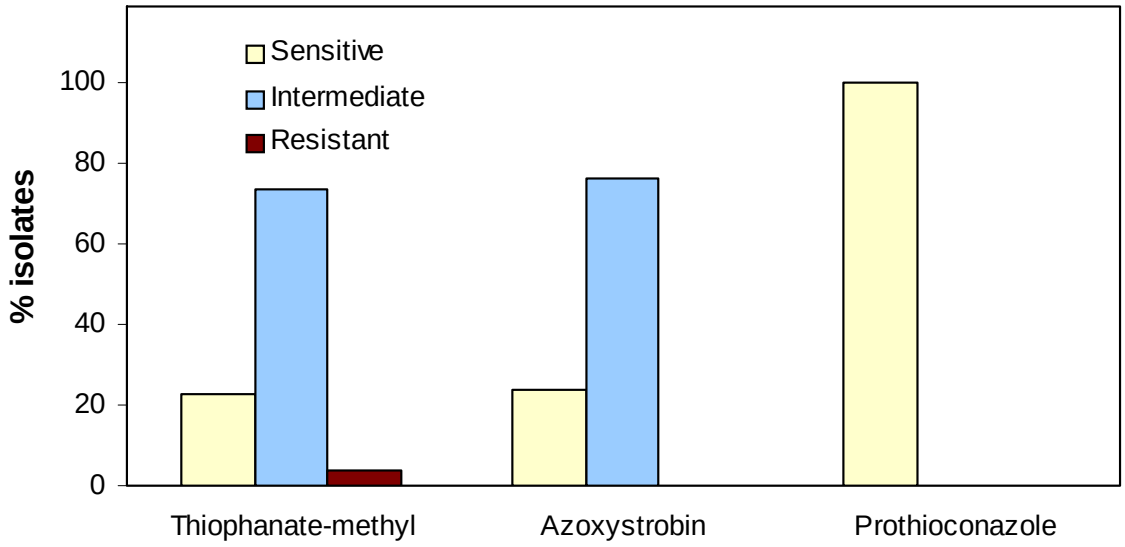

Fig. 3: Percentage of isolates resistant and sensitive to thiophanate-methyl, azoxystrobin, and prothioconazole (100 ppm) based on mycelial growth.

When determined based on spore production, 24% of the isolates were resistant to thiophanate-methyl, and 24% and 52% were sensitive and intermediately sensitive, respectively. 32% of the isolates were resistant to prothioconazole, and 52% and 16% were sensitive and intermediately sensitive to the product, respectively. 4% was resistant to azoxystrobin, and 52% and 44% were sensitive and intermediately sensitive, respectively (Figure 4).

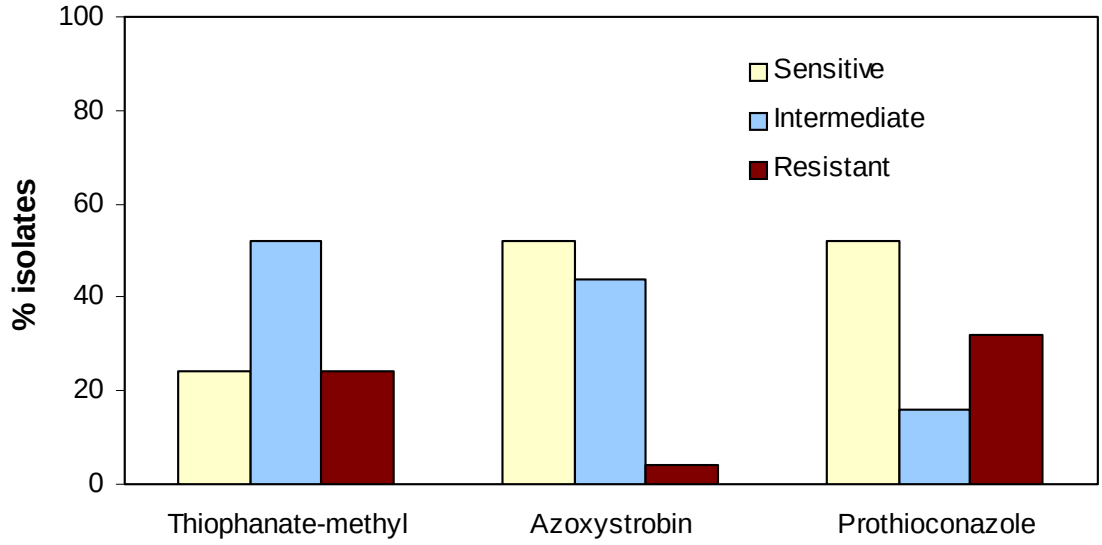

Fig. 4: Percentage of isolates resistant and sensitive to thiophanate-methyl, azoxystrobin, and prothioconazole (100 ppm) based on spore production.

Sensitivity to prothioconazole and azoxystrobin: EC_{50} values of prothioconazole ranged from 0.9 to 61.9 mg/liter, with an average of 5.7 mg/liter. EC_{50} values of azoxystrobin ranged from 1.9 to >1,000 mg/liter.

Conclusions

According to the studies on suppression of mycelial growth, some FON isolates from watermelon fields in Georgia and Florida have developed resistance to thiophanate-methyl. Isolates resistant to prothioconazole or azoxystrobin have not been found, and the isolates were more sensitive to prothioconazole than azoxystrobin. Inhibition of mycelial growth is critical in disease suppression since mycelial growth is essential in all infection processes of the pathogen. The studies also indicated that there were considerable differences among the FON isolates in their sensitivity to the three fungicides based on spore production. Using fungicides without resistance development in the pathogen populations (e.g. prothioconazole) and alternated applications of different fungicides are recommended for effective management of the disease.

References cited:

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2. Sanders, F. H., and Langston, D. B. 2011. Evaluation of selected fungicides for the control of Fusarium wilt and watermelon fruit blotch in Georgia, 2010. Plant Disease Management Reports 5:V156.