Monitoring development of fungicide resistance in populations of the Fusarium wilt pathogen

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Introduction

Fusarium wilt, caused by the fungal pathogen *Fusarium oxysporum* f. sp. *niveum* (FON), is one of the most damaging diseases of watermelon worldwide. It is a serious threat for watermelon production in the United States including Georgia. This pathogen causes pre- or post-emergence damping-off of young seedlings and greenhouse transplants, and death of seedlings is rapid under favorable conditions. In the field, symptoms typically occur within 3 to 4 weeks, starting with a graying of foliage followed by foliar chlorosis and wilt (Fig. 1). Plants affected late in the season may wilt and collapse or be stunted. Fusarium wilt is characterized by unilateral stem necrosis, which is easily visualized when runners or stems are sectioned.

Fig. 1. Fusarium wilt of watermelon caused by *Fusarium oxysporum* f. sp. *niveum.*

So far, four races of FON (0, 1, 2, 3) have been reported based on their ability to infect different watermelon genotypes. Our recent studies conducted at University of Georgia indicated that most of the FON isolates in Georgia belong to race 2 and race 3, two most aggressive races with no resistant watermelon cultivars available. Application of effective chemical fungicides is a recommended approach for developing integrated disease management strategies. However, fungicides for managing Fusarium wilt of watermelon are limited, and only prothioconazole (e.g., Proline) was registered for the disease. This narrow fungicide pool increases the risk of disease control failure due to potential fungicide resistance development. Prothioconazole belongs to demethylation inhibitor (DMI) fungicide group that targets a single site, the sterol 14α-Demethylase Cytochrome P450 (CYP51), an essential component of fungal membrane sterols required for proper membrane functioning. Development of resistance to fungicides with single site mode of action is common in populations of fungal pathogens, and current status of resistance of FON isolates to prothioconazole is unknown. The purpose of the project was to monitor development of resistance to prothioconazole in FON populations in Georgia. This information is crucial to guide growers to use effective fungicides to reduce losses caused by Fusarium wilt of watermelon.

Materials and Methods

FON isolates were collected from watermelon fields at different counties in Georgia. Pathogenicity of the isolates was verified by inoculation of a susceptible watermelon cultivar "Sugar Baby" under greenhouse conditions. The isolates were tested for sensitivity to

prothioconazole using traditional methods. Briefly, 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 at different concentrations ranging from 0 to 100 mg/liter (ppm). 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 fungicide (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; intermediately sensitive: 30 to 90% of the control; resistance: >90% of the control). The experiments were conducted twice under similar conditions. Percentage of resistant and sensitive isolates and fungicide concentrations that reduce 50% of the growth of selected representative isolates (EC_{50}) were calculated.

In order to simplify and shorten this process, a multi-well plate assay was evaluated. This assay tested sensitivity of several isolates to different fungicide concentrations on the same plate. Each well was filled with 1 ml of unamended potato dextrose broth (PDB, used as a control) or prothioconazole-amended PDB at 10 and 100 ppm. Suspensions of FON spores produced on agar plates were transferred to each well. After incubation at 25°C for 5 days, fungal growth was measured using a spectrophotometer.

Results

A total of 109 FON isolates were collected from different locations in Georgia. All isolates were sensitive to prothioconazole at 100 ppm and none of the isolates was resistant. At 10 ppm of prothioconazole, most of the isolates were sensitive, 14.7% isolates were intermediately sensitive, and no isolates were resistant (Fig. 2). Twenty representative isolates, 15 sensitive and 5 intermediately sensitive to 10 ppm of prothioconazole, were selected to determine fungicide concentrations that reduce 50% of the growth of the fungus (EC_{50}). EC_{50} values for the isolates ranged from 0.54 to 11.35 ppm (Fig. 3).

The results indicated that no isolates of FON in Georgia were found to be resistant to Proline (a.i., prothioconazole). However, some FON isolates showed reduced sensitivity to the fungicide compared to our previous studies. Hence integrated disease management programs are recommended to reduce selection pressure for FON populations to develop resistance to prothioconazole. In addition to the above studies on agar plates, a multi-well plate assay was used to assess sensitivity of FON isolates to prothioconazole by inoculating liquid medium in the wells with FON spores (200 spores/ml). The 20 representative isolates (15 sensitive and 5 intermediately sensitive) were tested and growth of the isolates was measured using a spectrophotometer 5 days after inoculation. At 100 ppm of prothioconazole, there was no significant difference among the isolates in OD (optical density) values. At 10 ppm of prothioconazole, OD values of the 15 sensitive isolates were significantly lower compared to the 5 intermediately sensitive isolates, indicating the assay could be used as a complementary method to determine sensitivity of FON isolates to the fungicide.

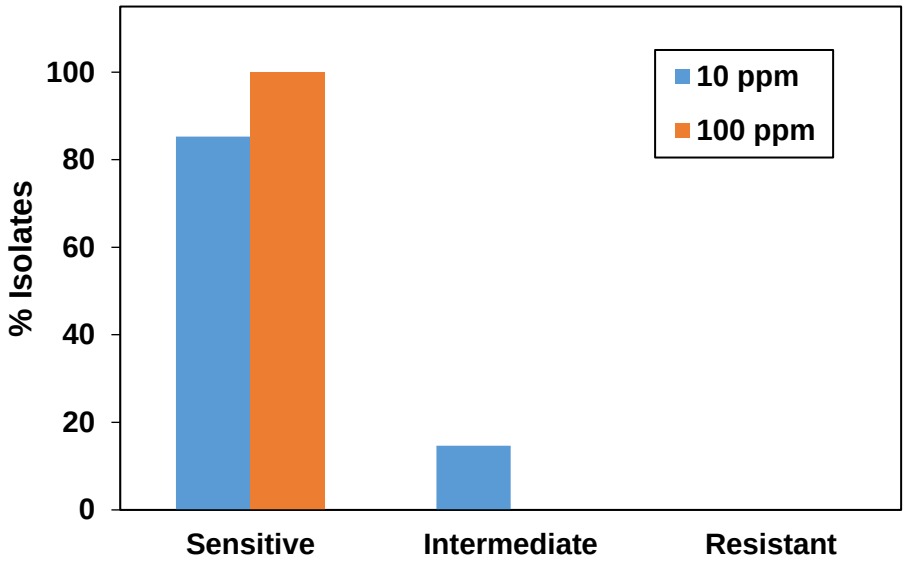

Fig. 2. Percentage of FON isolates that were sensitive, intermediately sensitive and resistant to prothioconazole.

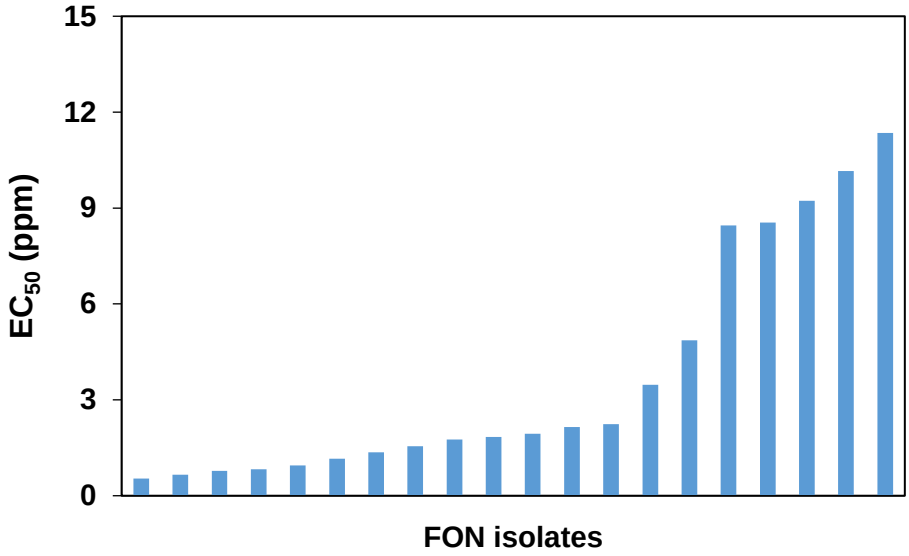

Fig. 3. Concentrations of prothioconazole that were 50% effective (EC_{50}) for inhibiting mycelial growth of *Fusarium oxysporum* f. sp. *niveum* (FON) isolates.