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August 28, 2018

Mr. Bob Morrissey
Executive Director
National Watermelon Association
5129 South Lakeland Drive, Suite 1
Lakeland, FL 33813

Dear Mr. Morrissey,

We are submitting two proposals entitled "*Monitoring development of fungicide resistance in populations of the Fusarium wilt pathogen*" and "*Development of integrated fungicide programs for managing Fusarium wilt of watermelon*" to National Watermelon Association for potential funding.

Georgia is among the top producers of watermelon in the nation. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* is a major disease in watermelon production in GA and surrounding states. Application of effective fungicides without resistance development in the pathogen population is critical to ensure successful reduction of losses caused by the disease. In one proposal, we will determine the distribution and percentage of pathogen isolates in GA that have developed resistance to prothioconazole, the only fungicide registered for the disease. In another proposal, we will evaluate and develop programs integrating fumigant (chloropicrin) and non-fumigant fungicides with different modes of action (prothioconazole, pydiflumetofen, and difenoconazole) for managing the disease.

The projects address the priority area "Fusarium wilt". Results of the projects will provide guidance to growers to use appropriate fungicides for control of Fusarium wilt of watermelon. Budgets of $10,000 and $8,000 are requested for the projects respectively.

Thanks for your consideration. I appreciate it if you could let me know in case additional information is needed.

Sincerely,

Pingsheng Ji

Pingsheng Ji
Professor

Project Title: Monitoring development of fungicide resistance in populations of the Fusarium wilt pathogen

Investigators:

Pingsheng Ji (Principal Investigator), Professor, Department of Plant Pathology, University of Georgia, Tifton, GA 31794. E-mail: pji@uga.edu

Emran Ali (Co-Principal Investigator), Director of Plant Molecular Diagnostic Lab, Department of Plant Pathology, University of Georgia, Tifton, GA 31794. E-mail: emran.ali@uga.edu

Justification for research:

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. 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.

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 so far only prothioconazole (e.g., Proline) is 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 isolates of FON may have developed resistance to prothioconazole during the frequent use of the fungicide in the past years. The purpose of the proposed project is to monitor development of resistance to prothioconazole in FON populations in Georgia. Our ultimate goal is to produce a map with location-specific resistance profiles to help understanding resistance development and spread. This information is crucial to guide growers to use effective fungicides to reduce losses caused by Fusarium wilt of watermelon.

Objectives/goals:

1) Conduct a statewide monitoring of resistance development in FON populations to prothioconazole.
2) Develop rapid and accurate methods for fungicide sensitivity evaluation.

Procedures:

FON isolates will be collected from watermelon fields at different locations (more than 10 counties) in Georgia. Pathogenicity of the isolates will be verified by inoculation of a susceptible watermelon cultivar under greenhouse conditions. The isolates will be 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 will be placed at the center of potato dextrose agar (PDA) plate amended with prothioconazole at different concentrations ranging from 0 to 100 mg/liter. Triplicate plates will be used for each isolate and the plates will be incubated at 25°C. Colony diameter will be 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 will be 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). The experiments will be conducted twice under similar conditions. Percentage of resistant and sensitive isolates and fungicide concentrations that reduce 50% of the growth of the fungus (EC_{50}) will be calculated.

Using the traditional methods to evaluate sensitivity to fungicides can be time-consuming and labor-intensive. In order to simplify and shorten this process, we will also develop a multi-well plate assay. This assay allows testing sensitivity of several fungal isolates to different fungicide concentrations on the same plate (Figure 1). Each well will be filled with 1 ml of unamended medium (control) or 1 ml of fungicide-amended medium.

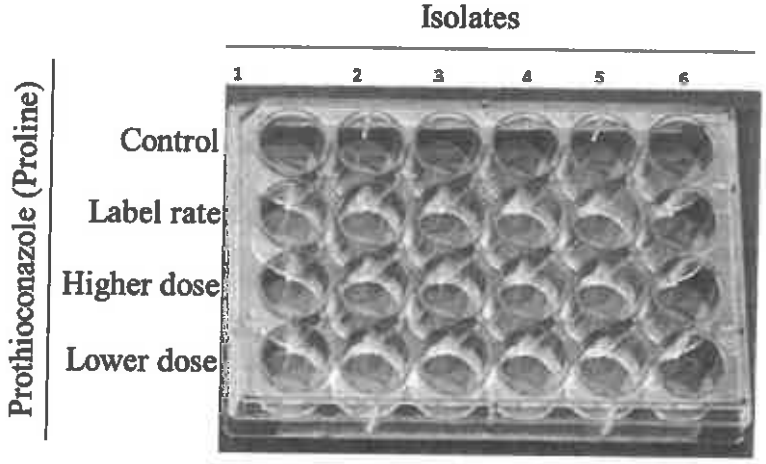

Figure 1. Multi-well plate assay (MWP) to evaluate sensitivity of FON isolates to prothioconazole.

For the multi-well plate assay, we will use suspensions of FON spores produced on agar plates that will be transferred to each well. On the fungicide side, we will use label rate and discriminatory doses determined in the studies above. After incubation at 25°C for 5 days, fungal growth will be measured using a spectrophotometer. The experiments will be repeated once and results will be compared with the traditional methods mentioned above.

Timeline: February 1, 2019 to January 31, 2020.

Dates for project report: September 2019 (intermediate) and January 2020 (final)

Date that Association will receive a final report: January 2020

Budget requested: $10,000

Personnel: $7,000 are requested for a part-time research personnel to help with the study.

Supplies: $3,000 are requested for lab and field sampling materials.

Priority area: Fusarium wilt

Addendum to proposal:

Project title: Monitoring development of fungicide resistance in populations of the Fusarium wilt pathogen

Lead Institution: University of Georgia

Investigators: Pingsheng Ji, Professor, University of Georgia
Emran Ali, Director of Plant Molecular Diagnostic Lab, University of Georgia

Project duration: February 1, 2019 to January 31, 2020

Budget requested: $10,000

Priority area: Fusarium wilt

Project summary:

Watermelon is a significant vegetable crop with more than 50% of the national production in the southeast. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *niveum* (FON) is a major yield limiting factor in watermelon production in Georgia and other southeastern states. Limited options are available for managing Fusarium wilt of watermelon and prothioconazole is the only fungicide registered for the disease. Application of effective fungicides without resistance development in the pathogen population is critical to ensure successful reduction of losses caused by the disease. In this project, FON isolates will be collected from different locations in Georgia, and distribution and percentage of FON isolates that have developed resistance to prothioconazole will be determined. In addition, a multi-well plate assay will be developed and validated for rapid and accurate evaluation of sensitivity of FON isolates to prothioconazole. The benefits of the project include providing guidance to growers to use appropriate fungicide without resistance development in the pathogen population and developing an efficient multi-well plate assay for fungicide resistance monitoring.