Title: Development of a regional fungicide resistance testing service for DMIs and QoIs using both conventional and molecular methods

Progress Report

Grant Code: SRSFC Project: #2020-08E (Category Service)

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Objectives: Provide fungicide resistance testing services to regional growers, extension specialists, and crop advisers

Justification and Description:
The University of Georgia Plant Pathology Department has established the Plant Molecular Diagnostic Laboratory (MDL) at the Tifton campus. This is a fee-based service lab, currently providing fungicide resistance testing support and routine advanced disease diagnosis to extension & research personnel, commercial growers & homeowners, for a wide range of plant pathogens. Fungicide resistance is a front-burner issue for growers and crop advisers over the last two to three decades. It can lead to lost disease control, reduced yields, and unnecessary expense by applying products that no longer work. A limited number of fungicide classes are available to manage fungal pathogens, and this narrow fungicide pool increases the risk of disease control failure due to potential fungicide resistance development. The DMI and QoI fungicides have a specific mode of action towards a target protein in fungal pathogens. A genetic adjustment by a fungus can lead to reduced sensitivity to these fungicides. The most important resistance mechanism is the modification of the fungicide target, caused by mutations in the encoding target gene and some cases by overexpression of the target gene. For the proper management of fungal pathogens, we need to have early, rapid, and accurate testing methods to identify fungicide resistance in various fungi. Unfortunately, there is no other single location in the Southeast that can provide resistance testing for multiple fungal organisms and multiple fungicides. This proposal will start to address that need. The overall goal of this proposed project is to optimize fungicide resistant testing for these fungicides at this lab and to establish a system

to provide support to growers and crop advisers. This service will be crucial to guide growers in the use of effective fungicides to reduce losses caused by fungicide resistance. This initial funding will provide an opportunity for fungal organisms to be tested for resistance development, especially where putative field failures have occurred. Initial testing will be limited to DMIs and QoIs, but additional fungicide classes and fungi will be added over time.

Procedures:

We have adapted the multi-well plate assay protocol from Dr. Guido Schnabel at Clemson University and transitioned their fungicide testing program to the molecular diagnostic laboratory at the University of Georgia. In brief for the MWP assay is mentioned below:

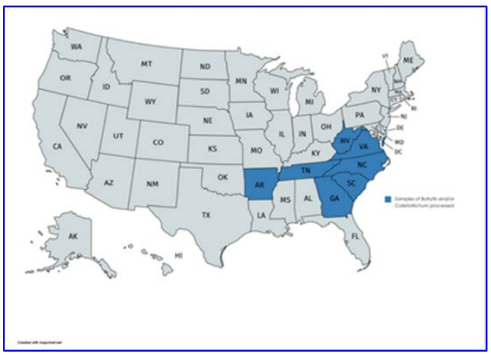
1. Samples from flowers, leaves, fruit, and cotton swabs with spores from fruit will be received for analysis as shown below:

Figure 1: Sample collection for the fungicide resistant testing

2. Suspected samples will be incubated for several days (depending on the pathogen's growth) in a moist chamber. This initial incubation process allows the pathogen to grow and sporulate on the sample surface.
3. Then the pathogen will be transferred onto the centers of fungicide-amended plates and nonamended control plates.
4. Microplates will be incubated for 5 days at 22°C before measuring the radial growth in two perpendicular directions and determining the pathogen's sensitivity to the respective fungicide.
5. Three micro-plates will be used for each fungicide and, sensitivity tests will be repeated twice. In general, this MWP process takes about ~7 days to take a final decision on fungicide sensitivity.
6. The molecular method will also be optimized based on target site mutation in relation to fungicide resistant phenotype. It is widely reported that resistance to quinone outside inhibitors (QoIs) has been associated with the presence of amino acid substitution G143A in the cytochrome b gene (Ali et al., 2019).

Current Progress on fungicide testing service to growers and crop advisers

In 2020, a total of 175 suspected strawberry samples were received from growers or extension county agents in six southern states and tested against different fungicide classes including DMIs, QoIs, and SDHIs at the MDL (Figure 1).



State	#Growers	#Samples
North Carolina	8	75
Virginia	2	30
South Carolina	2	20
Tennessee	1	10
Arkansas	2	20
Georgia	2	20
Total	**17**	**175**

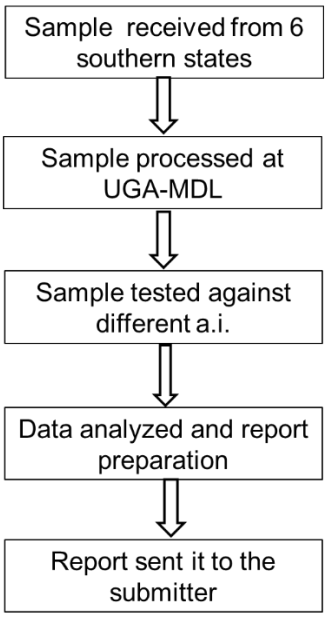


Figure 2: Sample collection map and layout for the fungicide resistant testing

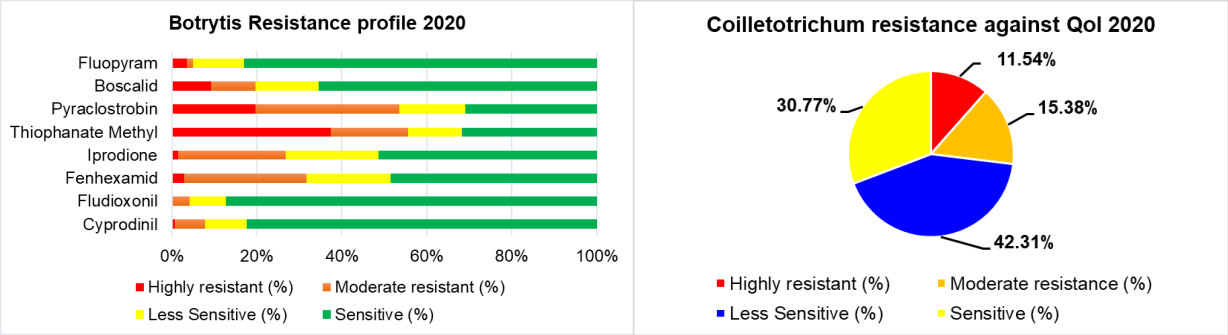


Figure 3: Fungicide resistance frequencies in 2020

The overall fungicide resistance frequencies were analyzed based on 145 *Botrytis* spp. isolated from six states and 30 *Colletotrichum* spp. isolates from three states. Our results showed that the efficacy of QoI (pyraclostrobin) fungicide is decreasing for controlling both *Botrytis* and *Colletotrichum* spp. isolates (Figure 3). Topsin M (thiophanate methyl) is also showing weakness due to the increasing resistance phenotype against *Botrytis* spp. (Figure 3).

Potential Impact

This service will be crucial to guide growers in the use of effective fungicides to reduce losses caused by fungicide resistance. Continuation of this testing service will provide management recommendations that will be valuable for small fruit growers in the southeastern U.S.

Literature Cited

1. Ali, E.M., Pandit, L.K., Mulvaney, K.A., Amiri, A. 2018. Sensitivity of Phacidiopycnis spp. Isolates from Pome Fruit to Six Pre- and Postharvest Fungicides. Plant Dis. 102(3):533-539.