<u>Progress Report</u>: North American Bramble Growers Research Foundation Grant 2020

Title: Prevalence and management of fungicide-resistant fungal pathogens of blackberry

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Introduction:

Fungal leaf spots and cane dieback are significant emerging disease issues for blackberry production in the southeastern US. Pseudocercospora leaf spot disease is widespread in Georgia blackberries, and can cause significant issues for producers, leading to premature defoliation and reduced plant vigor (**Fig. 1**). To control this disease, blackberry growers typically apply strobilurin or QoI (Quinone outside inhibitors – FRAC Group 11) fungicides including Abound, Cabrio, Quilt Xcel, and Pristine; however, in 2018, Pseudocercospora with QoI resistance was found to be causing severe issues on blackberry in North Carolina. Cane dieback is another emerging disease issue affecting blackberry production. Cane dieback in Georgia results from the infection of blackberry canes with several fungal pathogens including *Fusarium* sp., members of the Botryosphaeriacea family, and *Leptosphaeria coniothyrium*, cause of cane blight disease (Brannen and Krewer 2005). Pruning wounds are believed to serve as a primary entry point for these pathogens into the blackberry cane. After infection, canes can die back to the ground. Current recommendations for dieback control include the application of broad-spectrum fungicides, including Pristine. Nonetheless, in recent years, growers have reported significant issues with dieback despite prompt fungicide applications, and it is possible that fungicide-resistant pathogens may be playing a role in this emerging issue as well.

Fig. 1. Pseudocercospora leaf spot on blackberry.

Since blackberry growers in the southeastern US frequently rely on QoI and other fungicides for the management of disease issues, it is essential to assess whether fungicide resistance is widespread within commercial blackberry fields. Likewise, given the recent discovery of QoI-resistant *Pseudocercospora sp*., it is important that alternative fungicidal recommendations be established to help growers manage these issues. The current edition of the Southern Region Blackberry Management Guide recommends Tilt, Abound, Cabrio, Pristine, and Quilt Xcel for after harvest control of Pseudocercospora leaf spots, making Tilt the only non-QoI fungicide currently known to be effective against this disease (Oliver et al. 2019). Accordingly, to enhance management recommendations, it would be beneficial to assess the efficacy of additional fungicides such as newly registered members of the SDHI-class [Succinate dehydrogenase inhibitors - FRAC Group 7]. Therefore, the specific objectives of this work are: (1) Determine the prevalence of fungicide-resistant *Pseudocercospora* sp. and cane dieback

causing organisms in commercial blackberry plantings in Georgia, and (2) Evaluate fungicides for managing Pseudocercospora leaf spot in commercial blackberry production.

Methods:

Completed Work in 2020:

Isolation of fungal isolates from commercial blackberry

In September 2020, five blackberry plantings in Lanier, Bacon, and Pierce counties in southeastern Georgia were surveyed for the presence of leaf spot caused by *Pseudocercospora sp.* Symptomatic leaf tissue was collected in the field and transported to the UGA-Tifton Fruit Pathology Laboratory for fungal isolation. *Pseudocercospora sp.* were isolated from leaves using an adapted protocol for Cercospora culture (McClenning, unpublished). In brief, lesions were excised from leaves and surface sterilized using a series of four immersions in sterile water, 10% bleach, sterile water, and sterile water. Washed leaf lesions were then placed in a Petri dish containing 2 moist filter papers and incubated for 5-7 days until sporulation was evident. A sterile toothpick was used to remove the spores (conidia) and placed into a centrifuge tube containing 0.70 ml of sterile water. The water and spore solution was then decanted onto a 20% V8 agar plate (200 ml V-8 Juice, 800 ml distilled water, and 15 g agar) containing Streptomycin (0.27 mg/ml = 270 µg/ml) and Ampicillin (0.2 mg/ml = 200 µg/ml). Plates were incubated at 25°C with a 12 hr light/12 hr dark cycle. After one week, all colonies were transferred using sterile technique by cutting a small block (~1 cm x 1 cm) of the sporulating colony and blotting it onto a fresh V8 plate. Each block was blotted into an area approximately two to three times the size of the block to ensure adequate transfer of mycelia and spores. Plates were then incubated for approximately one week as described above to allow for fungal growth prior to DNA extraction.

Identification of fungal isolates

The identity of the cultured isolates was confirmed using morphological characteristics and sequencing. Fungal hyphae were collected from growing isolates for DNA extraction using a modified CTAB protocol (Doyle and Doyle 1987). The highly conserved ITS1 and ITS2 sequences flaking the 5.8S rDNA region were amplified by PCR with primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) (Martin and Rygiewicz 2005; White et al. 1990). Resulting amplicons were cleaned using an E.Z.N.A. Cycle Pure Kit (Omega Bio-tec, Inc., Norcross, GA), and sequenced via sanger sequencing by Eurofins Genomics (Louisville, KY). Resulting sequences for each isolate were compared to sequences in the Genbank NCBI database (National Center for Biotechnology Information, Bethesda, MD) using the BLASTn function. Sequences with a greater than 99% identity, greater than 99% query coverage, and an E-value of 0 to the best match sequence in Genbank were considered to belong to the same species. Following identification as *Pseudocercospora sp.*, selected isolates from each surveyed location were supplied to the UGA Plant Molecular Diagnostic Laboratory in Tifton, Georgia for fungicide resistance testing. Isolates were also saved for storage according to the methods described by Hemphill (2019). In addition, stocks of fungal isolates obtained previously from blackberry plants exhibiting dieback (Hemphill 2019) were also grown out for fungicide resistance screening.

Anticipated Work in 2021:

Screening fungal isolates for fungicide resistance

Fungal isolates from blackberry identified above will be tested for fungicide resistance by the UGA Plant Molecular Diagnostic Laboratory in Tifton, Georgia using a mycelial growth inhibition assay. Resistance to QoI fungicides will be assessed, as well as resistance to other fungicides including propiconazole (found in Tilt), boscalid (found in Pristine), fludioxonil & cyprodinil (found in Switch), and fluopyram (found in Luna Tranquility). Isolates found to be fungicide resistant will be genetically screened to determine if they possess known fungicide resistance mutations which may confer resistance to QoIs or other fungicide classes.

Field evaluation of fungicides for management of Pseudocercospora leaf spot

During the 2021 growing season, fungicides will be evaluated on blackberry cultivars 'Osage' and 'Ouachita' for control of Pseudocercospora leaf spot at a commercial blackberry site with a history of Pseudocercospora leaf spot disease. Four fungicides (Switch, Luna Tranquility, Miravis, and Tilt) (**Table 1**) and an untreated control will be compared for after-harvest control of Pseudocercospora leaf spot. Applications will begin ~14 days after harvest is complete and take place every 14-21 days. To directly compare fungicide efficacy, each treatment will consist of three applications of the same fungicide. Fungicides will be applied using a CO_2-powered backpack sprayer. Plots will consist of three adjacent bushes in the same row and a randomized complete block design will be used with five replications. Leaves will be collected from treated plots in late August and evaluated for disease severity (number of spots per leaf) and incidence (number of leaves with spots). Severity and incidence differences between treatments will be compared to determine fungicide efficacy.

Table 1. Fungicides for use in field trial.

Fungicide (Rate/Acre)	Active Ingredients	FRAC Group
Switch 62.5WG (14 oz)	cyprodinil+fludioxonil	9+12
Luna Tranquility (16 fl oz.)	fluopyram+pyrimethanil	7+9
Miravis (6.8 fl oz)	pydiflumetofen	7
Tilt (6 fl oz)	propiconazole	3

Results:

Pseudocercospora and cane dieback fungal isolates from blackberry

In total, 18 isolates were obtained from five commercial blackberry plantings in three counties in southeastern Georgia in 2020 (**Table 2**). All isolates were positively identified as *Pseudocercospora sp.* via morphological characteristics and sequencing. Five isolates representing each of the five surveyed blackberry plantings were

Table 2. Summary of *Pseudocercospora* isolates identified in 2020.

Site	County	Collection Date	Host	# of Isolates
Site 1	Lanier	9/2/2020	Blackberry cv. 'Ouachita'	4
Site 2	Lanier	9/2/2020	Blackberry cv. 'Osage'	4
Site 3	Lanier	9/2/2020	Blackberry cv. 'Caddo'	3
Site 4	Bacon	9/3/2020	Blackberry cv. 'Ouachita'	4
Site 5	Pierce	9/10/2020	Blackberry cv. 'Ouachita'	3

chosen for subsequent fungicide resistance screening. In addition, seven fungal isolates (**Table 3**) previously associated with cane dieback in Georgia blackberry plantings (Hemphill 2019) were also prepared from stocks for fungicide efficacy testing.

Table 3. Fungal isolates associated with blackberry cane dieback (Hemphill 2019).

Isolate ID	Species Identity	Collection County	ITS Accession Number
WH83	*Colletotrichum siamense*	Atkinson	MN718940
WH3	*Fusarium oxysporum*	Irwin	MN718865
WH131	*Lasiodiplodia pseudotheobromae*	Dougherty	MN718987
WH30	*Lasiodiplodia theobromae*	Oglethorpe	MN718891
WH22	*Neofusicoccum kwambonambiense*	Oglethorpe	MN718884
WH45	*Neofusicoccum parvum*	Lanier	MN718906
WH17	*Pestalotiopsis microspora*	Oglethorpe	MN718879

Summary:

To better understand the prevalence of fungicide-resistant fungal pathogens of blackberry in Georgia and options for their management, this proposal consisted of two objectives: (1) Determine the prevalence of fungicide-resistant *Pseudocercospora sp.* and cane dieback causing organisms in commercial blackberry plantings in Georgia, and (2) Evaluate fungicides for managing Pseudocercospora leaf spot in commercial blackberry production. The objectives of this project were originally anticipated to be completed over two years with Objective 1 completed in 2020 and Objective 2 completed in 2021. As a result of UGA mandated restrictions on laboratory and field research due to the ongoing COVID-19 pandemic, the fungicide resistance screening of fungal isolates collected in 2020 as a part of this work was delayed and results are not available as of the date of this report. Fungicide resistance screening of Pseudocercospora isolates collected in 2020 is currently underway, and screening of cane dieback fungi is expected in January 2021. Field work anticipated in 2021 as part of Objective 2 is expected to be completed as scheduled. A final report summarizing this work is anticipated in December 2021.

References:

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