<u>Progress Report</u>: North American Bramble Growers Research Foundation Grant 2018

Title: Evaluation of fungicides for management of cane blight disease of blackberry

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Justification and Description:

Blackberries (*Rubus* sp.) are a valuable and popular small fruit crop; however, blackberry production faces numerous challenges. In particular, many diseases result in high yield losses. Among these, cane diseases, including cane blight, can be significant and potentially result in complete destruction of the fruiting canes (Brannen and Krewer 2005) (Figure 1). The fungal pathogen *Leptosphaeria coniothyrium* is recognized as the causal agent of cane blight disease. Outside of the US, cane blight is not generally reported as a major disease of blackberries except where winter injury occurs on thornless blackberries, and much of the currently available information on the management and biology of cane blight comes from research done on machine-harvested red raspberries in Scotland (Williamson 2017). Infection with the cane blight fungus occurs through wounds resulting from cane injury via pruning, machinery, insect damage, freeze damage, herbicide damage, or infection with other pathogens. Visible symptoms of cane blight include the formation of lesions on primocanes and floricanes; these can ultimately grow together, girdling the cane and resulting in cane death (Brannen and Krewer 2005).

Figure 1. Cane blight symptoms including dead/dying canes with a silvery or gray appearance. Damage may be associated with pruning cuts. Photo by E. Smith.

As a whole, knowledge of the epidemiology and management of this disease is lacking. Though fungicide applications after pruning are recommended, very little is known about which fungicides are most efficacious to use. While blight can be severe, the irregularity with which cane blight occurs complicates field experiments investigating chemical management options. A routine greenhouse and field inoculation system would facilitate the more rapid validation of new disease management practices, but little work has been done previously in this regard, except to show that fungal mycelium alone, in the absence of wounding, only slightly damages canes (Williamson 2017). Therefore, we proposed to develop a system utilizing fungal isolates collected from blighted canes to compare the efficacy of fungicides on cut surfaces via a routine inoculation system. The ultimate goal of this work is to provide additional cane blight management tools and recommendations for blackberry growers.

Proposed Objectives:

1) Isolate and confirm *L. coniothyrium* fungal isolates associated with blighted canes for use in greenhouse and field studies.
2) Develop an inoculation system to artificially infect canes with fungal isolates associated with cane blight.
3) Utilize collected isolates to test the efficacy of fungicides against cane blight on cut surfaces in both greenhouse and field settings.

Anticipated Timetable:

The objectives of this project are expected to be completed over two years: Objective 1 in 2018; Objective 2 in 2018-2019; and Objective 3 in 2019. A final report is expected to be delivered in December 2019.

Methods:

Fungal isolation and storage

From commercial blackberry plantings in four Georgia counties (Table 1), surveys were conducted in fall ('17), spring ('18), and summer ('18) to identify fungal isolates associated with cane blight. Diseased canes exhibiting symptoms of cane blight were sampled for fungi, and fungal isolates were grown out and established in pure culture. For long-term storage of isolates for use in subsequent experiments, each isolate was grown out on plates of quarter-strength PDA (Difco) acidified with lactic acid (AqPDA) and sterilized filter paper pieces. The colonized filter paper pieces were removed, placed in sterilized and labeled coin envelopes, and allowed to dry in a laminar flow hood. Once dry, envelopes were sealed, bound together, added to a plastic container with desiccant pellets, and placed at -20°C for long-term storage.

Fungal isolate identification

Each isolate obtained above was subsequently identified genetically. To identify each isolate, DNA was extracted from individual pure cultures using the 5% Chelex protocol (UCLA 2002). With the extracted DNA used as a template, PCR was carried out with the primer pair ITS-1 (TCCGTAGGTGAACCTGCGG) and ITS-4 (TCCTCCGCTTATTGATATGC). The PCR conditions included an initial activation step at 95°C for five minutes which was followed by 35 repeats of a denature step at 95°C for 30 seconds, an annealing step at 55°C for 30 seconds, and an extension step at 72°C for 30 seconds. This was followed by a final extension step at 72°C for 30 seconds. Resulting PCR products were visualized on a 1.5% agarose gel, and the PCR product was purified for sequencing using the QIAquick® PCR Purification Kit (Germantown, MD). Sanger sequencing was carried out by Eurofins Genomics (Louisville, KY), and resulting sequences were analyzed phylogenetically using Geneious® software (v 11.0.4) versus known sequences obtained from Genbank (ncbi.nlm.nih.gov). Maximum sequence identities ≥95% were used to identify isolates to genus, and ≥97% sequence identities were used to identify the species of isolates (Table 2).

Plant materials

Potted plant experiments were carried out on blackberry plants received in February 2018. These consisted of 'Ouachita' blackberry plants derived from meristem tissue culture

(North American Plants, Inc.). Outdoor plants were grown in Sta-Green® Potting Mix Plus Fertilizer (Sims Bark Co., Inc., Muscle Shoals, AL) in 7.7 gal pots. These were fertilized with Osmocote® Plus 15-9-12 (The Scotts Company, LLC, Maryville, OH) and maintained outside on ground cover cloth under daily overhead irrigation. Plants in the greenhouse were grown in 2 gal pots filled with Miracle-Gro® Moisture Control Potting Mix (The Scotts Company, LLC, Maryville, OH). These were maintained at 26°C during the day and 22°C at night, fertilized with Osmocote Plus 15-9-12, and watered daily as needed.

Isolate pathogenicity testing and artificial inoculation of blackberry

To verify the ability of obtained fungal isolates to cause disease on blackberry, identified fungal isolates were used to inoculate cut canes of potted blackberry plants. Fungal isolates were grown on AqPDA at room temperature (~23°C) and agar plugs containing fungal mycelium from the growing edge of the colony were removed from the plates after one week. Each plug was removed with a 7 mm sterilized cork borer and placed inside a 1.5 mL microcentrifuge tube. Tubes were inverted onto the cut ends of blackberry primocanes and secured with parafilm to provide a moist chamber for fungal infection. After seven days, the parafilm and microcentrifuge tubes were removed from the cut cane.

Potted blackberry plants (outdoors) were inoculated with unique fungal isolates in two experiments in a randomized complete block design. In each experiment, three plants received each isolate and three primocanes on each plant were inoculated. The first experiment lasted six weeks from 6/18/2018 to 7/30/2018 and consisted of 12 treatments including nine fungal isolates and three control treatments (Table 3). The second experiment lasted eight weeks from 8/29/2018 to 10/25/2018 and consisted of 16 treatments including 13 fungal isolates and three control treatments (Table 4). Cane dieback (in mm) from the cane tip was measured weekly starting 7 or 9 days post-inoculation in the first and second experiment, respectively. Disease progress (cane dieback) was summarized over the course of the experiment as AUDPC. At the conclusion of the experiment, cane dieback caused by each isolate was compared using a generalized linear mixed modeling analysis in PROC GLIMMIX in SAS v. 9.4 (SAS Institute, Cary, NC). Isolates capable of causing significant dieback were noted for use in subsequent experiments.

Planned Work:

Fungicide efficacy testing

Fungicide efficacy testing will be carried out in the greenhouse and field. The following treatments will be used to test the efficacy of fungicides for cane blight management: (1) Abound, (2) Pristine, (3) Rally, (4) Tilt, (5) Captan, (6) Switch, (7) Topsin M, and (8) ProPhyt, (9) untreated control, and (10) uninoculated control. For the greenhouse work, in addition to testing fungicide efficacy, the effect of timing between pruning, fungicide application, and fungal inoculation will also be investigated. Therefore, fungicides will be applied to cut surfaces either 24 hours before, within a few hours of, or 24 hours after inoculation with the fungus. For all trials, five potted blackberry plants (cv. 'Ouachita') will receive each treatment with three cut primocanes per plant. Field experimental work will be carried out in a commercial blackberry planting. Following pruning and fungal inoculation, treatments will be applied in a randomized complete block design. Five replications of each treatment will be conducted, and each replication will consist of

a single plant. After plants are pruned, fungicide applications in the field will be made with a backpack sprayer to runoff. A minimum of one plant will be skipped between spray plants to minimize plot-to-plot spray drift. Variables measured will be disease incidence and lesion length, and treatments means will be compared.

Results:

Fungal isolate collection and identification

Canes showing dieback typical of cane blight were collected in 2017 and 2018 from commercial blackberry plantings in four Georgia counties: Irwin, Lanier, Berrien, and Oconee. From collected canes, 47 unique isolates were obtained (Table 1) and identified genetically (Table 2). Surprisingly, none of the collected isolates were identified as *Leptosphaeria coniothyrium*, the recognized causal agent of cane blight; however, numerous isolates from fungal genera (*Fusarium*, *Pestalotiopsis*) and families (Botryosphaeriaceae) known to include plant pathogens were identified (Table 2). This suggests the possibility that other fungi found in commercial blackberry plantings in Georgia may be capable of causing symptoms approximating those typical of cane blight.

Table 1. Number of fungal isolates collected by county of origin.

County (in GA)	Number of Isolates
Berrien	2
Irwin	4
Lanier	21
Oconee	20

Table 2. Isolate counts corresponding to each identified species.

Family	Species	Counts
Botryosphaeriaceae	*Diplodia seriata*	7
	Lasiodiplodia theobromae/hormozganensis/brasiliensis	3
	Neofusicoccum kwambonambiense	2
	Neofusicoccum parvum	2
Nectriaceae	*Fusarium oxysporum*	5
	Fusarium proliferatum	2
	Fusarium acuminatum	1
	Fusarium chlamydosporum	1
Pestalotiopsidaceae	*Pestalotiopsis clavispora*	5
	Pestalotiopsis microspora	4
	Neopestalotiopsis clavispora	2
	Neopestalotiopsis chrysea	1
Glomerellaceae	*Colletotrichum gloeosporioides*	3
Gilbertellaceae	*Gilbertella persicaria*	2
Diaporthaceae	*Diaporthe hongkongensis*	1
Didymellaceae	*Phoma herbarum*	1
Pleurostomataceae	*Pleurostoma richardsiae*	1
Schizophyllaceae	*Schizophyllum commune*	1
Sporidiobolaceae	*Sporidiobolus pararoseus*	1
Microascales incertae sedis	*Sphaeronaemella fragariae*	2

Isolate pathogenicity testing and artificial inoculation of blackberry

The pathogenicity of collected isolates was assessed on cut blackberry canes in two experiments. In the first experiment, an isolate of *Fusarium oxysporum* was the only organism to cause significant cane dieback (Table 3) through the six-week experiment. On average, plants inoculated with this isolate showed 56 mm dieback at 6-weeks post-inoculation. Intriguingly, it was noted that by 13 weeks post-inoculation, the inoculated canes of all three plants that received this isolate had dieback to the crown and over 95% of the canes were dead (Figure 3). All three plants were completely dead to the crown at 15 weeks post-inoculation.

Table 3. Cane dieback in the first isolate pathogenicity experiment.

Identity of Isolate Used for Inoculation	**AUDPC[z]**
Fusarium oxysporum	2326.33 a[y]
Pestalotiopsis clavispora	926.33 b
Phoma herbarum or Leptoshaeria sacchari	893.67 b
Neopestalotiopsis clavispora	825.42 b
Sporidiobolus pararoseus	679.00 b
Fusarium proliferatum	638.75 b
Uninoculated Control (Cut cane with agar and tube)	633.50 b
Colletotrichum gloeosporioides	616.00 b
Pestalotiopsis microspore	447.42 b
Diplodia seriata	422.33 b
Control (Cut cane with no tube)	290.00 b
Control (Uncut cane)	n/a

[z]Average cane dieback (AUDPC) over the 3 replicates.
[y]Means followed by same letter are not significantly different (at *P*=0.05).

Figure 3. (A) Plant inoculated with the *F. oxysporum* isolate from the first isolate pathogenicity experiment at 12-weeks post-inoculation, (B) uninoculated plant at 12-weeks post-inoculation.

In the second experiment, four isolates (*Pestalotiopsis microspora, Neofusicoccum kwambonambiense, Lasiodiplodia theobromae, and Neofusicoccum parvum*) caused significant cane dieback (Table 4) through the eight-week experiment. Cane dieback caused by these isolates by week eight was, on average, 54.67 mm, 57.22 mm, 49.63 mm, and 52.67 mm, respectively. It must be noted that the isolate of *Fusarium oxysporum* from pathogenicity experiment one did not exhibit a statistically significant amount of disease in pathogenicity experiment two. All isolates shown to be capable of causing significant disease on cut canes will be retested on greenhouse-grown blackberry and in the fungicide efficacy trials in 2019.

Table 4. Cane dieback in the second isolate pathogenicity experiment.

Identity of Isolate Used for Inoculation	**AUDPC[z]**	
Neofusicoccum kwambonambiense	1951.80	a[y]
Lasiodiplodia theobromae/hormozganensis/brasiliensis	1643.65	a
Neofusicoccum parvum	1611.14	a
Pestalotiopsis microspore	1400.00	a
Fusarium acuminatum	434.42	b
Fusarium oxysporum	424.84	b
Pleurostoma richardsiae	391.28	b
Gilbertella persicaria	209.23	b
Diplodia seriata	184.23	b
Uninoculated Control (Cut cane with agar and tube)	153.43	b
Fusarium chlamydosporum	151.65	b
Schizophyllum commune	129.09	b
Diaporthe hongkongensis	114.73	b
Fusarium oxysporum	104.99	b
Control (Cut cane with no tube)	51.67	b
Control (Uncut cane)	n/a	

[z]Average cane dieback (AUDPC) over the 3 replicates.
[y]Means followed by same letter are not significantly different (at *P*=0.05).

References:

Brannen, P. and Krewer, G. 2005. Cane Blight of Blackberry. University of Georgia Extension Circular 894. http://extension.uga.edu/publications/detail.html?number=C894

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