Title: Investigating the prevalence of QoI-resistant anthracnose fruit rot on blueberry in Georgia and evaluating fungicides for disease management

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Public Abstract

For blueberry producers, fruit rots including Botrytis fruit rot, Anthracnose ripe rot, Alternaria fruit rot, and Phomopsis fruit rot cause significant losses. Anthracnose fruit rot of blueberry, caused by the fungi *Colletotrichum acutatum* and *C. gloeosporioides*, results in a destructive rot of ripe fruit called ripe rot that can affect fruit either before or after harvest. In commercial blueberry production, anthracnose fruit rot is typically managed through multiple applications of fungicides from bloom until harvest. Strobilurin or QoI (Quinone outside inhibitors) fungicides have been effective for ripe rot management, and blueberry growers in the southeastern US have relied heavily on QoI fungicides including Pristine and Abound for ripe rot control. However, in recent years, growers and packing houses have reported increasing problems with anthracnose, and *Colletotrichum sp.* isolates with resistance to QoI fungicides have recently been identified in blueberries in Florida, South Carolina, and Georgia. To determine the prevalence of QoI-resistant *Colletotrichum sp.* in Georgia and provide growers with alternative fungicidal recommendations, survey work and field trials were initiated in commercial blueberry fields in Georgia. Specifically, fungicide programs were evaluated for their effectiveness at controlling anthracnose fruit rot and other fruit rot issues on blueberry. Based upon the field trial results, fungicide programs incorporating Switch, Captan, Ziram, Omega, and/or Miravis Prime were the most successful at reducing the incidence of fruit rots in general and ripe rot specifically. Survey work to determine the prevalence of QoI-resistant *Colletotrichum sp.* is expected to be completed during 2021.

Introduction

Anthracnose fruit rot of blueberry is caused by the fungi *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* (Milholland 2017). This disease affects both southern highbush (SHB) and rabbiteye blueberries in the southeastern United States. Though this disease can also affect blossoms and shoots, it is primarily known for causing a destructive rot of ripe fruit called ripe rot. Ripe rot can affect fruit either before or after harvest, and can cause significant losses when warm, wet weather occurs during bloom or just prior to harvest. Though infection with ripe rot fungi can occur as early as bloom, symptoms typically don't appear until fruit ripens. On infected fruit, salmon-colored spore masses can be produced (**Figure 1)** which allow the fungus to easily infect other fruit on the plant or other fruit in the same packing line or clamshell. As a result, ripe rot can cause significant losses in blueberry production. Typically, field losses due to ripe rot increase in successive pickings, and in poor storage conditions postharvest losses can approach 100%. In the southeastern United States, anthracnose also causes a damaging leaf spot as well, and severe disease can result in premature defoliation.

Figure 1. Salmon-colored sporulation of the ripe rot fungus (*Colletotrichum sp.*) on blueberry. Photo from Miles and Schilder 2008.

In commercial blueberry production, anthracnose fruit rot is managed through multiple applications of fungicides from bloom until harvest, with after-harvest applications used to manage anthracnose leaf spot. DMI (Demethylation Inhibitor) fungicides have proven to be relatively poor in their control of anthracnose fruit rot, while strobilurin or QoI (Quinone outside inhibitors) fungicides have been effective. Accordingly, blueberry growers in the southeastern US have relied heavily on QoI fungicides including Pristine and Abound, as well as broad-spectrum fungicides like Captan, for ripe rot control. However, in recent years, growers and packing houses have reported increasing problems with anthracnose, and *Colletotrichum sp.* isolates with resistance to QoI fungicides have been identified in blueberries in Florida (Phillips et al. 2018) and South Carolina (Hu et al. 2015). In 2019, three isolates of *C. gloeosporioides* were obtained from ripe fruit in two SHB blueberry fields in Pierce County, Georgia (Ali et al. 2019). These fields had been noted to have especially severe issues with ripe rot, despite repeated applications of QoI fungicides. The three isolates obtained were found to be highly resistant to pyraclostrobin and possess the G143A amino acid substitution in the cytrochrome b gene previously associated with QoI resistance in *Colletotrichum sp.* (Forcelini et al. 2018). These three isolates notwithstanding, prevalence of QoI resistant anthracnose in blueberries in Georgia remains largely unknown.

Since blueberry growers in Georgia frequently rely on QoI fungicides for the management of ripe rot, it is essential to assess how widespread QoI-resistant *Colletotrichum sp.* are within commercial blueberry fields. Likewise, given the recent discovery of QoI-resistant *C. gloeosporioides* in Georgia, it is important that alternative fungicidal recommendations be established to help growers manage ripe rot. The current edition of the Southern Region Blueberry

Integrated Management Guide (https://smallfruits.org/ipm-production-guides/) recommends Abound, Pristine, Quilt Xcel, Switch, Captan, and Omega for ripe rot control; however, Omega is rarely used due to its long pre-harvest interval (30 days), while Abound, Pristine, and Quilt Xcel all include QoI fungicides. That leaves only Captan and Switch from this list in common use, and Switch is frequently relied upon for Botrytis management during bloom with the total applications per season limited by the label. Accordingly, to enhance ripe rot management recommendations, it would be beneficial to assess additional fungicides for their efficacy versus ripe rot including newly registered members of the SDHI-class [Succinate dehydrogenase inhibitors - FRAC Group 7]. Therefore, we evaluated fungicide programs for managing anthracnose fruit rot in southern highbush blueberry plantings and plan to conduct a survey of commercial blueberry plantings in southeastern Georgia for the presence of QoI-resistant *Colletotrichum sp.*

Materials and Methods

Evaluation of fungicide programs for managing anthracnose fruit rot in southern highbush blueberry plantings. Chemicals were evaluated for control of fruit rot in two locations in Bacon and Pierce counties in Georgia in 2020. The first trial was carried out in a planting of SHB blueberry cultivar 'Star' at the Blueberry Research Farm in Alma, GA in Bacon County. The second trial was conducted in a planting of SHB cultivar 'Farthing' at a commercial blueberry farm near Blackshear, GA in Pierce County. In both locations, treatments were applied at 30% bloom (5 Feb), petal fall (19 Feb), 10 days after petal fall (6 Mar), ~3 weeks after petal fall (17 Mar), and pre-harvest (25 Mar). All treatments were applied until runoff (equivalent to 75 gal water/A) using a Solo backpack sprayer with an 8003 TeeJet spray tip. Chemical treatments consisted of five spray programs: (1) Switch 62.5WG and Captan Gold 4L, (2) Switch 62.5WG, Captan Gold 4L, and Miravis Prime (3) Switch 62.5WG, Captan Gold 4L, and Luna Tranquility, (4) Switch 62.5WG, Ziram, and Omega 500F, and (5) Switch 62.5WG, Ziram, Miravis Prime, Omega 500F, and Luna Tranquility (**Table 1**). Five replications of each treatment and the untreated control were applied to a randomized complete block design, with each plot consisting of three sprayed plants, with one unsprayed plant separating each plot. All cultural practices were consistent with southern highbush blueberry production methods commonly observed in the Southeast.

Table 1. Spray programs evaluated for fruit rot control.

	Application Timings[z]				
Program	**#1**	**#2**	**#3**	**#4**	**#5**
1	Switch	Captan	Switch	Captan	Switch
2	Switch	Captan	Miravis	Captan	Miravis
3	Switch	Captan	Luna Tranquility	Captan	Luna Tranquility
4	Switch	Ziram	Switch	Omega	Switch
5	Switch	Ziram	Miravis	Omega	Luna Tranquility
Untreated Control	n/a	n/a	n/a	n/a	n/a

[z]The five application timings correspond to (1) 30% bloom, (2) petal fall, (3) 10 days after petal fall, (4) ~3 weeks after petal fall, and (5) pre-harvest.

For trial evaluation, ripe fruit were harvested on 21 Apr from the Bacon County location and on 24 Apr and 14 May from the Pierce County location. Approximately 150-200 fruit were collected from the center plant in each plot and placed into a plastic clamshell container for transport and storage. Harvested fruit were stored for 36-48 hrs at room temperature and then evaluated for rot. Fruit was evaluated for marketability. Soft, leaky, or sporulating berries were considered unmarketable. Rots were evaluated based on visual signs.

Survey of commercial blueberry plantings in southeastern Georgia for the presence of fungicide-resistant *Colletotrichum sp.* [***Note: Due to research and travel restrictions related to the ongoing COVID-19 pandemic, the collection of ripe fruit and laboratory analyses necessary to isolate and identify fungal organisms as a part of this work were not able to be performed during the 2020 harvest season (April-June 2020). It is anticipated that this work will instead be performed during 2021 as UGA travel and research restrictions allow.****] During the 2021 growing season, approximately ten commercial blueberry plantings within the southeastern Georgia blueberry production region (Appling, Bacon, Pierce, Ware, and Clinch counties) will be surveyed for the presence of QoI-resistant *Colletotrichum sp.* Plantings will be chosen based upon their past history of ripe rot or current season ripe rot issues as reported by growers and UGA Cooperative Extension personnel. Ripe blueberry fruit will be collected from up to five plants per site and returned to the UGA Fruit Pathology Laboratory in Tifton, Georgia for pathogen isolation. Acidified quarter strength potato dextrose agar (AqPDA) will be used for isolation, and the identity of the cultured isolates will be confirmed using morphological characteristics and sequencing of the ITS1 and ITS4 fragment (White et al. 1990). All obtained *Colletotrichum* isolates will subsequently be tested for QoI fungicide resistance by the UGA Plant Molecular Diagnostic Laboratory in Tifton, Georgia using a mycelial growth inhibition assay (Ali et al. 2018). Resistance to additional fungicides may also be assessed as funds allow.

Results

Fungicide programs significantly reduce anthracnose fruit rot and other rots on harvested blueberries. At both locations, conditions were adequate for disease development with a significant percentage of fruit harvested from the untreated control plots showing evidence of rot (**Figure 2**). At the Bacon County location, over 17% of the untreated control fruit showed evidence of rot at the time of evaluation, and at the Pierce County location, over 15% of the untreated control fruit showed rot (**Table 2**). With respect to anthracnose fruit rot specifically, more ripe rot was observed at the Pierce County location (over 7% of the untreated fruit) relative to the Bacon County location (2% of the untreated fruit). All programs resulted in a significantly lower incidence of ripe rot versus the untreated control except for program 3 consisting of Switch 62.5WG, Captan Gold 4L, and Luna Tranquility.

Figure 2. Harvested blueberries showing anthracnose fruit rot and other rots. Photo by Jonathan Oliver.

With respect to all rots (*Colletotrichum sp.*, *Phomopsis vaccinii*, *Alternaria tenuissima*, and other unidentified fungi), program 2 consisting of Switch 62.5WG, Captan Gold 4L, and

Miravis Prime resulted in a significantly lower incidence versus the untreated control at both locations. In addition, programs 1, 4, and 5 also resulted in a significantly lower incidence of all rots in the Pierce County location only (**Table 2**). No phytotoxicity was observed at any time following treatment applications.

Table 2. Fruit rot field trial results summarized from both locations.

			Ripe Rot (%)[y]		All rots (%)[x]	
Program	**Treatment and amount/A**	**Application timing[z]**	**Pierce[v]**	**Bacon[w]**	**Pierce[v]**	**Bacon[w]**
	Untreated control	----	7.1 a	2.0 a	15.7 a	17.3 a
1	Switch 62.5WG 14 oz	1,3,5	1.9 b	0.4 b	7.0 b	11.7 ab
	Captan Gold 4L 2.5 qts	2,4				
2	Switch 62.5WG 14 oz	1	3.3 b	0.1 b	8.1 b	4.1 b
	Captan Gold 4L 2.5 qts	2,4				
	Miravis Prime 13.4 oz	3,5				
3	Switch 62.5WG 14 oz	1	6.7 a	1.1 ab	15.6 a	11.2 ab
	Captan Gold 4L 2.5 qts	2,4				
	Luna Tranquility 16 fl oz	3,5				
4	Switch 62.5WG 14 oz	1,3,5	1.4 b	0.1 b	4.8 b	13.4 ab
	Ziram 3 lbs	2				
	Omega 500F 1.25 pts	4				
5	Switch 62.5WG 14 oz	1	1.7 b	0.3 b	5.7 b	6.9 ab
	Ziram 3 lbs	2				
	Miravis Prime 13.4 oz	3				
	Omega 500F 1.25 pts	4				
	Luna Tranquility 16 fl oz	5				

[z]Treatments were applied at (1) 30% bloom (5 Feb), (2) petal fall (19 Feb), (3) 10 days after petal fall (6 Mar), (4) ~3 weeks after petal fall (17 Mar), and (5) pre-harvest (25 Mar).

[y]Rot caused by *Colletotrichum sp.* Identified based upon visual observations. Means in each column followed by the same letter are not significantly different according to the least significant difference test (LSD)($\alpha = 0.05$).

[x]Rots caused by *Colletotrichum sp.*, *Phomopsis vaccinii*, *Alternaria tenuissima*, and other unidentified fungi. Means in each column followed by the same letter are not significantly different according to the least significant difference test (LSD)($\alpha = 0.05$).

[v]Recorded for ~150-200 fruit collected on 24 Apr and on 14 May.

[w]Recorded for ~150-200 fruit collected on 21 Apr.

Survey of commercial blueberry plantings in southeastern Georgia for the presence of fungicide-resistant *Colletotrichum sp.* As indicated above, this survey could not be carried out in 2020 due to travel and research restrictions imposed following the COVID-19 pandemic. Results from this survey are anticipated in 2021.

Discussion

Based upon the results of the field trial, fungicide programs incorporating Switch, Captan, Ziram, Omega, and/or Miravis Prime were the most successful at reducing the incidence of fruit rots in general and ripe rot specifically. This is in agreement with the ripe rot efficacy ratings provided by the 2020 Southern Region Blueberry Integrated Management Guide (https://smallfruits.org/ipm-production-guides/), for Switch ('Excellent'), Omega ('Good'), Captan ('Good'), and Ziram ('Fair'). Miravis Prime has not been previously recommended in this guide, however, based partially upon these trial results, it will be included in the 2021 edition of this guide with a rating of 'Very Good' for ripe rot control. By contrast, based upon the relatively poor performance of spray program 3 in this trial (which included two applications of Luna Tranquility), Luna Tranquility is not recommended for control of ripe rots or other fruit rots at this time. Taken together, these results suggest that QoI-resistant ripe rot can be managed using a rotation of the above effective products, and Georgia blueberry growers will be encouraged to incorporate these fungicides into their spray program during the bloom to pre-harvest period. A survey for fungicide-resistant *Colletotrichum sp.* will be carried out in 2021, and information on the prevalence of QoI-resistant *Colletotrichum sp.* within commercial blueberry fields in Georgia will be used to further enhance ripe rot management recommendations for growers.

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