Title: Investigating the prevalence of fungicide resistance among fungi causing Alternaria and Botrytis fruit rots on Georgia blueberries

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Public Abstract

Fruit rots, including Botrytis gray mold and Alternaria fruit rot, can cause significant losses in commercial blueberry production. To reduce losses, growers typically apply chemical fungicides. Unfortunately, the fungal organisms that cause fruit rots (including *Botrytis cinerea* and *Alternaria* spp.) can readily develop resistance to many of the single-site fungicides most frequently used in commercial agriculture. If prevalent, fungicide-resistant pathogens have the potential to reduce or eliminate the effectiveness of many of the most important fungicides used for managing fruit rots in blueberry production systems. Unnecessary (ineffective) fungicide applications and significant financial losses can be the result for growers if spray programs are not adjusted to account for the presence of fungicide resistance. To investigate the prevalence of fungicide-resistance among fungi causing fruit rots in Georgia blueberry fields, isolates of *Alternaria* and *Botrytis* spp. were collected from symptomatic blueberry fruit during the 2021 and 2022 harvest seasons and screened for fungicide resistance. Fungicide resistance testing and genetic analysis of these isolates indicated the presence of resistance to pyraclostrobin and boscalid among *Alternaria* isolates and resistance to multiple fungicides among the screened *Botrytis* isolates. These results reinforce the importance of good fungicide resistance management practices going forward. Furthermore, they suggest that Georgia blueberry growers experiencing fruit rot control failures may benefit from fungicide resistance monitoring to ensure that the fungicides they are relying on for control will be effective against the fungal isolates present in their respective fields.

Introduction

While many plant diseases impact blueberry production systems, fruit rots can be especially devastating. This is because losses due to fruit rots often do not become apparent until harvest time – exactly when all the hard work put into the crop is expected to soon pay off. While there are numerous fruit rot diseases of blueberry that can affect fruit either pre- or postharvest, three of the most significant are Anthracnose fruit rot (caused by *Colletotrichum sp.*), Botrytis fruit rot or gray mold (caused by *Botrytis cinerea*), and Alternaria fruit rot (caused by *Alternaria spp.*) (**Figure 1**). These fungal fruit rots can routinely cause significant pre- and postharvest losses in

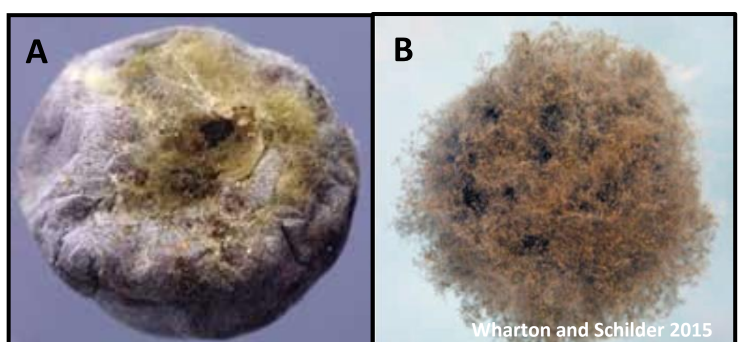

Figure 1. (A) Alternaria and (B) Botrytis fruit rots.

blueberry production systems if unmanaged (Bristow et al. 2017, Milholland and Cline 2017). Accordingly, in-field disease management practices (spray programs) as well as harvesting and handling recommendations largely focus on preventing losses from these rots (Sial et al. 2023).

During the past three growing seasons, Georgia blueberry growers have reported significant losses due to fruit quality issues in multiple locations. While the reasons for the observed problems with fruit quality are not fully understood, significant issues with fungal fruit rots have been observed in at least some locations – even in fields where regular applications of heretofore effective fungicides have been made. One explanation for this may be the presence of fungicide resistance within the pathogen populations at some of these locations, and indeed *Colletotrichum spp.* with resistance to QoI fungicides (quinone outside inhibitors; FRAC Group 11) have been isolated from at least one location in Georgia during this time (Ali et al. 2019).

To further investigate the reported fruit quality issues, the UGA Fruit Pathology Laboratory in Tifton conducted a survey of fungal fruit rots in 2021 and 2022. Through this work, fungal isolates were cultured from rotting blueberry fruit collected from multiple locations throughout the southeastern part of Georgia. In total, 836 fungal isolates were cultured and identified from 46 blueberry plantings in six southeastern Georgia counties over the 2021 and 2022 growing seasons (with the bulk of these isolates having been collected during the 2021 growing season). Included in this collection were numerous isolates of *Alternaria* spp. and *Botrytis cinerea* (**Table 1**).

Table 1. *Botrytis* and *Alternaria* isolates collected from rotting blueberry fruit during 2021 & 2022.

Fungal Species	2021 Fruit Rot Survey Isolates Collected [32 locations]	2022 Fruit Rot Survey Isolates Collected [8 locations]	Totals
Botrytis cinerea	155	12	167 isolates
Alternaria spp.	47	8	55 isolates

On blueberry, *Alternaria spp.* and *Botrytis cinerea* cause Alternaria fruit rot and Botrytis fruit rot, respectively (Bristow et al. 2017, Milholland and Cline 2017). These diseases, like other fruit rots, are frequently managed in commercial blueberry production through the use of fungicide applications from bloom through harvest (Sial et al. 2023). Unfortunately, the pathogens causing these diseases are also well-known to develop resistance to many of the fungicides commonly used for management (Saito et al. 2016, Wang et al. 2022) especially the single-site fungicides used for controlling these diseases (**Table 2**).

Table 2. Single-site fungicides used for control of Botrytis and Alternaria fruit rots in blueberries.

Treatment	Active Ingredients	FRAC MoA	Efficacy vs. Alternaria*	Efficacy vs. Botrytis*
Switch	cyprodinil+fludioxonil	9+12	Excellent	Excellent
Pristine	pyraclostrobin+boscalid	11+7	Excellent	Excellent
Quilt Xcel	azoxystrobin+propiconazole	11+3	Excellent	N/A
Abound	azoxystrobin	11	Excellent	N/A
Quash	metconazole	3	Excellent	N/A
Elevate	fenhexamind	17	N/A	Excellent
Omega	fluazinam	29	Good	Fair

*Ratings from the 2023 SE Regional Integrated Management Guide for Blueberries (Sial et al. 2023).

If prevalent, fungicide-resistant pathogens have the potential to reduce or eliminate the effectiveness of many of the most important fungicides used for managing fruit rots. Unnecessary (ineffective) fungicide applications and significant financial losses can be the result for growers if spray programs are not adjusted to account for the presence of fungicide resistance. Unfortunately, there had previously been little to no information available in Georgia on the fungicide-resistance status of *Alternaria* and *Botrytis* isolates from blueberry. As such, and given the observed issues with fruit rots in Georgia over the past several growing seasons (even in fields where presumably adequate spray programs were utilized), the objective of this work was to screen isolates of *Alternaria spp.* and *Botrytis cinerea* collected from rotting blueberry fruit in Georgia during the 2021 and 2022 growing seasons for resistance to fungicides commonly used to manage blueberry fruit rot diseases.

Materials and Methods

Fungicide resistance screening. Isolates of *Botrytis cinerea* and *Alternaria spp.* collected from blueberries during the 2021 and 2022 growing seasons were selected and screened for fungicide resistance at the UGA-Tifton Fruit Pathology Laboratory. Stocks of each isolate were previously prepared on filter paper and placed at -20°C for long-term storage, and all isolates were previously identified based on morphological characterization and genetic sequencing of the fungal ITS1 and ITS4 fragment (White et al. 1990). Fungicide resistance screenings with each isolate were carried out in duplicate using a previously described (Ali et al. 2018) mycelial growth inhibition assay (**Figure 2**). For *Alternaria* isolates, the fungicides cyprodinil, fludioxonil, pyraclostrobin, boscalid, metconazole, and fluazinam were utilized for screens. For Botrytis isolates, the fungicides cyprodinil, fludioxonil, pyraclostrobin, boscalid, fenhexamid, and fluazinam were utilized for screens. Based on the results of these assays, the prevalence of fungicide resistance amongst all screened isolates and amongst all collection locations (blueberry farms) was determined.

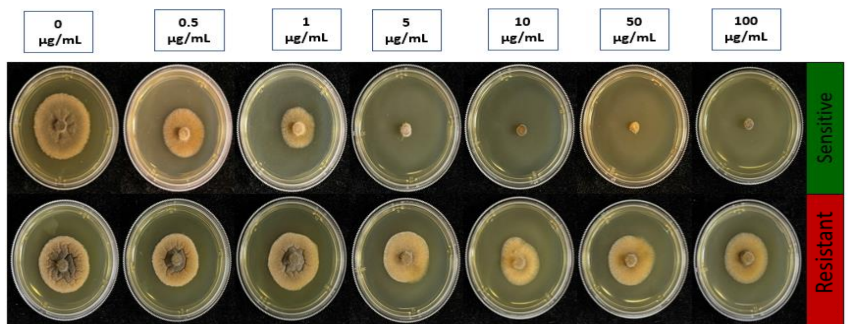

Figure 2. An example of a mycelial growth inhibition assay. A different fungal isolate was used for each row of plates. Each column represents a different concentration of fungicide. The isolate on the top row is sensitive to the fungicide, whereas the isolates on the bottom row is fungicide resistant. Photos by Mirza Beg, UGA.

Fungicide resistance confirmation using genetic analysis. Mutations within the cytochrome b gene in some fungi have been previously shown to confer resistance to all QoI fungicides (including pyraclostrobin) (Fisher and Meunier 2008); mutations in the *erg27* gene of *Botrytis cinerea* have been associated with resistance to fenhexamid (Grabke et al. 2013); and mutations within the succinate dehydrogenase genes (*sdhB*, *sdhC*, and *sdhD*) have been associated with resistance to SDHI fungicides (including boscalid) in some fungi (Landschoot et al. 2017). For isolates identified as being resistant to these fungicides, additional genetic testing and sequencing was performed as appropriate for confirmation.

Results

Fungicide resistance identified. In total, 46 *Alternaria* isolates (16 locations in 4 counties) and 117 *Botrytis* isolates (19 locations in 3 counties) were screened for fungicide resistance. *Alternaria* isolates with resistance to pyraclostrobin and/or boscalid and *Botrytis* isolates with resistance to pyraclostrobin, cyprodinil, fludioxonil, and/or fenhexamid were identified (**Table 4**). Six of 46 (13%) *Alternaria* isolates [3 of 16 sites] were found to be resistant to BOTH components of Pristine (pyraclostrobin+boscalid). In addition, 5 of 117 (4%) *Botrytis* isolates [4 of 19 sites] were found to be resistant to BOTH components of Switch (cyprodinil+fludioxonil), and 1 *Botrytis* isolate was identified with resistance to both Switch (cyprodinil+fludioxonil) and Elevate (fenhexamid).

Table 4. Fungicide screening results and prevalence of resistance among tested locations.

Fungicide Active (FRAC #)		**Alternaria**			**Botrytis**		
		Field Efficacy[1]	**Resistant Isolates[2]**	**Locations w/Resistance**	**Field Efficacy[1]**	**Resistant Isolates[2]**	**Locations w/Resistance**
Pristine	pyraclostrobin (11)	Excellent	10 of 46 (22%)	6 of 16 (38%)	Excellent	12 of 117 (10%)	9 of 19 (47%)
	boscalid (7)		21 of 46 (46%)	6 of 16 (38%)		10 of 117 (9%)	6 of 19 (32%)
Switch	cyprodinil (9)	Excellent	All Sensitive	0 of 16 (0%)	Excellent	30 of 117 (26%)	11 of 19 (58%)
	fludioxonil (12)		All Sensitive	0 of 16 (0%)		7 of 117 (6%)	6 of 19 (32%)
Elevate	fenhexamid (17)	n.r.	not tested	not tested	Excellent	7 of 117 (6%)	7 of 19 (37%)
Omega	fluazinam (29)	Good	All Sensitive	0 of 16 (0%)	Fair	All Sensitive	0 of 19 (0%)
Quash	metconazole (3)	Excellent	All Sensitive	0 of 16 (0%)	n.r.	not tested	not tested

[1]Field efficacy is based on the 2023 Southeast Regional IPM Guide. (Excellent, Very Good, Good, Fair, Poor, or Not Recommended [n.r.])
[2]Red = # (%) of Fungicide Resistant Isolates, Orange = # (%) of Moderate Resistance, Green = All Isolates are Sensitive to Fungicide

Genetic analysis supports fungicide resistance screening results. While genetic analysis is still underway as of the time of this report, mutational analysis of *Alternaria* isolates suggested that all pyraclostrobin-resistant isolates possessed the G143A mutation in the cytochrome b gene and are therefore likely to be resistant to other QoI fungicides as well (Abound & one component of Quilt Xcel). In addition, mutations with the succinate dehydrogenase genes (*sdhB*, *sdhC*, and *sdhD*) from boscalid-resistant *Alternaria* isolates also suggest that these isolates are likely to be resistant to other SDHIs (pydiflumetofen [1 component of Miravis Prime] and fluopyram [1 component of Luna Tranquility]). Analysis of mutations in fungicide-resistant *Botrytis* isolates is currently in progress, but some mutations have already been identified in the *erg27* gene of fenhexamid-resistant *B. cinerea* isolates from this study.

Discussion

By determining if fungicide-resistant isolates of *Botrytis* and *Alternaria* are present in Georgia blueberry fields, growers can be provided with valuable information regarding the likely effectiveness of particular fungicides. If fungicide-resistance is widespread in a given location, growers can experience disease control failures when utilizing those fungicides to which resistance has developed. Through the knowledge regarding fungicide resistance provided by this research, growers will be able to make more informed decisions regarding fungicide choices, reducing the use of ineffective fungicides and improving control of fruit rots on blueberry.

This work helped to confirm the presence of fungicide-resistant *Alternaria* and *Botrytis* spp. in Georgia blueberries for the first time. For *Alternaria*, no resistance was identified to any of the other tested fungicides besides pyraclostrobin and boscalid (both found in Pristine). This suggests that other fungicides used for fruit rot control are still expected to provide efficacy against Alternaria fruit rot at this time. For *Botrytis*, a relatively low percentage of isolates (~10% or less) were identified with resistance to most of the tested fungicides besides cyprodinil (one component of Switch). This result suggests that in most locations and situations, control of Botrytis gray mold can still be achieved through the use of good fungicide resistance management practices. Accordingly, tank mixes with multi-site fungicides (Captan or Ziram) are strongly recommended for fruit rot control, and rotations with different fungicide modes of actions remain essential to reduce the chances of further resistance development. The fact that all *Alternaria* and *Botrytis* isolates tested in this work were sensitive to the fungicide fluazinam (found in Omega) coupled with this fungicide's known efficacy against fruit rots ("Good" for Alternaria and "Fair" for Botrytis) suggests that this fungicide may be useful in fungicide rotation programs for fruit rot control going forward.

As a whole, this work highlights the importance of fungicide resistance monitoring in blueberries. Georgia growers who have experienced fruit rot control failures may benefit from fungicide resistance analysis to ensure that the fungicides they are relying on for control will be effective against the fungal isolates present in their respective fields. Growers desiring to have their fields/fruit tested for fungicide-resistant fruit rot pathogens are encouraged to contact the UGA Plant Molecular Diagnostic Laboratory in Tifton.

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