Title: Identifying post-harvest rots of Georgia blueberries and assessing pre-harvest fungicide applications for prevention of post-harvest diseases

Progress Report: Funded Research Proposal

Grant Code: SRSFC Project #2021 R-13

Name, Mailing and E-mail Address of Principal Investigator(s)

Principal Investigator:	Co-Principal Investigator:
Jonathan E. Oliver	Emran Ali
Assistant Professor	Assistant Research Scientist
Dept. of Plant Pathology	Department of Plant Pathology
UGA – Tifton Campus	UGA – Tifton Campus
2360 Rainwater Road	2360 Rainwater Road
Tifton, GA 31793-5737	Tifton, GA 31793-5737
jonathanoliver@uga.edu	emran.ali@uga.edu

Public Abstract

Many fruit rot diseases, including Botrytis fruit rot, Anthracnose ripe rot, and Alternaria fruit rot, affect blueberry fruit. In the field, these diseases are generally managed with fungicide applications throughout the season, however, the fungal pathogens that cause these diseases can also cause significant fruit rot in clamshells and in packing lines after harvest. In addition, numerous other fungal organisms can cause post-harvest fruit rots on blueberry and lead to significant losses. During the 2020 growing season in particular, Georgia rabbiteye blueberry growers reported significant post-harvest fruit quality issues due to yeast rot (caused by the fungus *Aureobasidium pullulans*). As a result of the losses experienced during the 2020 growing season, blueberry growers in Georgia are eager for information regarding options for reducing post-harvest fruit rot issues and prolonging the shelf life of blueberries after harvest. To better understand what organisms are primarily responsible for post-harvest fruit rots in Georgia blueberries, and to determine if fungicide applications prior to harvest can be effective for reducing post-harvest fruit rot issues and prolonging the shelf life of blueberries after harvest, field trials and survey efforts were carried out during the 2021 blueberry growing season. Surveys of harvested blueberry fruit showing signs of post-harvest rots resulted in the isolation and identification of numerous fungal organisms, and work is ongoing to determine which of these organisms are capable of causing post-harvest fruit rots under standard storage conditions. The field trials examined the effects of fungicides applied immediately prior to harvest (preharvest) both on fruit quality at harvest and on the subsequent development of post-harvest fruit rots during cold storage of harvested fruit. Results indicated little to no impact of preharvest fungicide applications on fruit rots present at harvest; nonetheless, a significant reduction in post-harvest rot development in cold storage was noted on fruit harvested from trial plots treated with some of the fungicide utilized in these trials. Since it takes time to sort, pack, and ship blueberry fruit prior to the fruit reaching market, these trial results suggest that fungicide applications prior to harvest may be of benefit in order to preserve fruit quality after harvest.

Introduction

Fruit rots can be devastating to blueberry production. Botrytis fruit rot (caused by the fungus *Botrytis cinerea*), Anthracnose ripe rot (caused by the fungal species *Colletotrichum gloeosporioides* and *C. acutatum*), Alternaria fruit rot (caused by the fungus *Alternaria tenuissima*), and Phomopsis fruit rot (caused by the fungus *Phomopsis vaccinii*) are important diseases that regularly impact blueberry production worldwide (Bristow et al. 2017; Milholland 2017; Milholland and Cline 2017; Milholland and Schilder 2017). These diseases are prevalent in blueberry production systems in Georgia (Mehra et al. 2013), and targeted sprays from bloom through harvest are necessary to manage these disease issues in commercial production. If unmanaged, infection with the fungal organisms causing these diseases can lead to significant pre- and post-harvest rots, and significant losses, on a regular basis. In addition to these organisms, many other fungal organisms can cause post-harvest fruit rots on blueberry, albeit with less frequency (Wharton and Schilder 2015). Among these infrequent causes of post-harvest fruit rot is the organism that causes yeast rot.

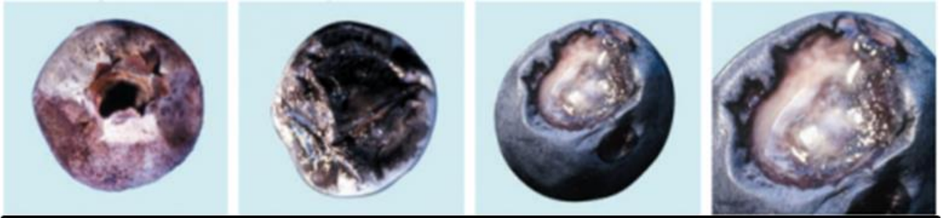

Figure 1. Progression of yeast rot (caused by *Aureobasidium pullulans*) on blueberry. Images from the Michigan Blueberry Facts: Fruit Rot Identification Guide (E2847).

Yeast rot is a sporadic post-harvest rot of blueberries that is rarely reported (Wharton and Schilder 2015). Fruit affected by yeast rot rapidly collapse and take on a wet, slimy appearance (**Figure 1**). Yeast rot is caused by the fungus *Aureobasidium pullulans*, a ubiquitous fungus known to live naturally on or within numerous plant species (Cook 1959). This fungus readily colonizes fruit surfaces, especially wounds on fruit, and is sometimes considered a secondary or weak pathogen that can cause yeast rot of blueberry fruit under certain conditions. Like many of the less frequent post-harvest fruit rot causing organisms, chemical management options for yeast rot are largely unknown, though it is conceivable that fungicides applied immediately prior to harvest may reduce the development of post-harvest fruit rots.

During the latter part of the 2020 blueberry growing season in Georgia (late May and early June), several growers and packing houses began reporting severe problems with fruit quality and post-harvest fruit rots. In particular, yeast rot was diagnosed repeatedly on ‘rabbiteye’ fruit harvested from multiple locations, and as a result, packing houses in Georgia were forced to reject numerous loads of harvested fruit. The issues were so severe that several packing houses closed early for the season due to the lack of available quality fruit. The financial losses due to this were severe, and many rabbiteye growers abandoned their harvests completely, resulting in an estimated 35-40% loss of the entire rabbiteye harvest from Georgia in 2020. The exact causes of these reported issues aren’t completely clear, but it is theorized that a strong environmental component was involved. In particular, excessive rainfall and hot temperatures immediately prior to the rabbiteye harvest were likely key conditions leading to the observed issues. Excessive rainfall events prior to harvest are known to lead to fruit splits and wounds. Since the fungal organism that causes yeast rot is known to colonize wounds, it is probable that excessive rainfall and perhaps the warm temperatures resulted in fruit splits and excessive colonization of rabbiteye blueberry fruit with the yeast rot fungus prior to harvest. This ultimately may have resulted in the observed post-harvest disease issues.

As a result of the significant losses sustained in 2020 due to post-harvest fruit quality issues, blueberry growers in Georgia are desperate for information regarding options for reducing post-harvest fruit rot issues and prolonging the shelf life of blueberries after harvest. To better understand the organisms causing post-harvest fruit rots on Georgia blueberries and determine if pre-harvest fungicide applications could help growers to reduce issues with post-harvest fruit rots, we conducted research with the following objectives: (1) Identify organisms causing post-harvest fruit rots on Georgia blueberries, and (2) Assess the potential of pre-harvest fungicide applications to reduce the incidence of post-harvest fruit rots and prolong the shelf life of harvested blueberries.

Materials and Methods

Identification of organisms causing post-harvest fruit rots on Georgia blueberries. During the 2021 growing season, fruit was collected from commercial blueberry plantings within six counties in southeastern Georgia (Appling, Bacon, Brantley, Clinch, Pierce, and Ware). A composite sample of ripe blueberry fruit was collected from up to five plants per site (100-200 fruit per plant) and returned to the UGA Fruit Pathology Laboratory in Tifton, Georgia for pathogen isolation. Acidified quarter strength potato dextrose agar (AqPDA) was used for isolation, and purified isolates were placed on filter paper for storage in coin envelopes at -20°C. The identity of the cultured isolates was confirmed using morphological characteristics and sequencing of the ITS1 and ITS4 fragment (White et al. 1990).

While *this work is still in progress at the time of this report*, a representative subset of obtained isolates will subsequently be assessed for their ability to cause post-harvest rots on ripe blueberry fruit under standard storage conditions. Ripe blueberry fruit (either purchased commercially or obtained from packing houses prior to shipment) will be surface disinfested and inoculated with fungal isolates. Inoculated fruit (in clamshells) will be placed in refrigerated storage conditions typically used for the preservation of harvested fruit and monitored daily for the development of fruit rot for up to three weeks. Fruit will be monitored for visible signs of berry leakage, softness, and/or fungal growth. Following this assessment, fungal organisms will have been identified that are: (1) associated with ripe rabbiteye and southern highbush blueberry fruit in Georgia and (2) capable of causing post-harvest fruit rot under standard storage conditions.

Assessment of the potential of pre-harvest fungicide applications to reduce the incidence of post-harvest fruit rots and prolong the shelf life of harvested blueberries. The effectiveness of preharvest fungicide applications at reducing post-harvest fruit rot was evaluated in four commercial sites in Pierce and Bacon counties in Georgia during the 2021 growing season. Two commercial rabbiteye (RE) blueberry sites (both growing cultivar 'Brightwell') and two commercial southern highbush (SHB) blueberry sites (cultivars 'Meadowlark' and 'Farthing') were used for application trials. Both rabbiteye locations had experienced significant losses and yeast rot during 2020. The application trials evaluated labelled fungicides with short pre-harvest intervals which represented several different FRAC Groups (**Table 1**). The short pre-harvest intervals associated with these fungicides allowed them to be applied in the field close to harvest.

Table 1. Fungicides used in field trial.

Fungicide	Rate/Acre	Active Ingredients	FRAC Group	Pre-harvest Interval
Elevate	1.5 lb	fenhexamid	17	0 days
Abound	15.5 oz	azoxystobin	11	0 days
Switch 62.5WG	14 oz	cyprodinil+fludioxonil	9+12	0 days
Luna Tranquility	16 fl oz	fluopyram+pyrimethanil	7+9	1 day
Miravis Prime	6.8 fl oz	pydiflumetofen+ fludioxonil	7+12	0 days

Since the focus of this trial was to specifically evaluate the impact of fungicide applications made immediately prior to harvest, all plants were subjected to the standard fungicide application program utilized by the grower at each respective location up until the first treatment associated with this trial. Each treatment for this trial consisted of two consecutive applications with the same fungicide. The first application was made at the 5-10% blue fruit stage (approximately 7-14 days prior to the first harvest) and the second application was made at mid-harvest (approximately 7-10 days after the first harvest). Six treatments (the five fungicides listed in **Table 1** and an untreated control) were applied to a randomized complete block design with five replications at each location. Plots consisted of three adjacent bushes in the same row, and fungicides were applied using a CO_2-powered backpack sprayer. At the conclusion of the trial, a few days after the final fungicide application, ripe fruit (150-200) was hand-harvested from the center plant of each plot and placed into a plastic clamshell container and transported to the UGA-Tifton Fruit Pathology Laboratory for evaluation.

Post-harvest fruit rot evaluation. At the UGA-Tifton Fruit Pathology Laboratory, harvested fruit was evaluated for post-harvest fruit rot development in two portions. To assess the effect of different fungicidal treatments on the longevity/shelf-life after harvest, one portion of the harvested fruit from each plot (in clamshells) was placed into refrigerated storage conditions and monitored daily for 3-5 weeks for the development of fruit rot. Fruit was evaluated for marketability. Soft, leaky, or sporulating berries were considered unmarketable. The second portion of the harvested fruit from each plot was initially stored for 36-48 hours at room temperature and then evaluated for rot incidence on the basis of visual signs. Selected fruit showing different types of rot were utilized for fungal isolation and identification (as described above).

Results

Identification of organisms causing post-harvest fruit rots on Georgia blueberries. In total, 781 fungal isolates were obtained from fruit collected in 31 plantings in six southeastern Georgia counties. Fruit were collected from at least 4 rabbiteye cultivars (‘Alapaha’, ‘Brightwell’, ‘Powderblue’, and ‘Premier’) and at least 12 SHB cultivars (‘Emerald’, ‘Farthing’, ‘Georgia Dawn’, ‘Indigocrisp’, ‘Keecrisp’, ‘Meadowlark’, ‘Optimus’, ‘Patrecia’, ‘Rebel’, ‘Star’, ‘Suziblue’, and ‘V1’). At the time of this report, 146 out of the 781 isolates originally collected have been sequenced for identification (**Figure 2**). *Due to the*

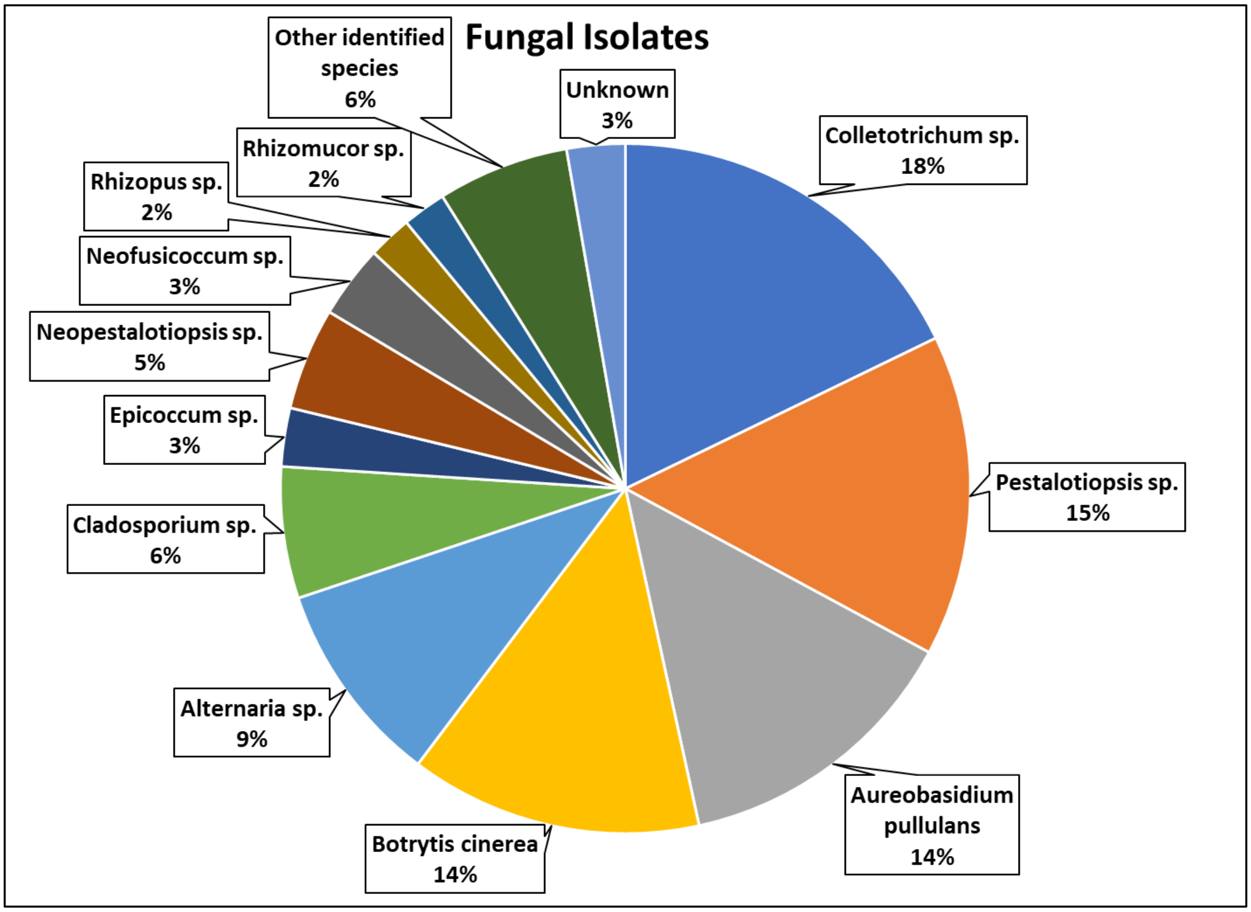

Figure 2. Fungal isolates identified from post-harvest fruit rot survey during 2021. Results from the first 146 identified isolates (out of 781 total fungal isolates) are shown. Following the conclusion of the fungal identification, a representative subset of obtained isolates will subsequently be assessed for their ability to cause post-harvest rots on ripe blueberry fruit under standard storage conditions.

large number of isolates that are being processed, this effort is expected to carry over into 2022, and will likely be completed during spring 2022.

Evaluation of fruit after harvest (field trial results). The evaluation of fruit after harvest did not indicate substantial fungicide treatment effects. In fact, only one treatment (Miravis Prime) at a single site (the Pierce rabbiteye site) was found to result in a significant reduction in rot (**Table 2**) relative the untreated control. An exceptionally large number of fruit (nearly 50% of untreated control fruit) harvested from the rabbiteye sites in Bacon and Pierce County showed significant evidence of rot after 48 hours, but relatively little ripe rot (Anthracnose fruit rot) was observed at any of the trial locations.

Table 2. After harvest fruit rot evaluation results from all four field trial locations.

		All rots (%)[yx]				Ripe Rot (%)[wx]			
		SHB		**Rabbiteye**		**SHB**		**Rabbiteye**	
Treatment and amount/A	**Application timing[z]**	**Bacon**	**Pierce**	**Bacon**	**Pierce**	**Bacon**	**Pierce**	**Bacon**	**Pierce**
Untreated control	----	9.2 a	12.4 a	51.8 a	47.4 a	0.8 a	1.1 a	0.0 a	1.2 a
Elevate 1.5 lb	1, 2	6.3 a	12.2 a	44.0 a	40.8 ab	0.3 a	0.7 a	0.0 a	1.2 a
Abound 15.5 fl oz	1, 2	6.6 a	12.6 a	35.3 a	43.5 a	0.4 a	0.4 a	0.0 a	0.3 a
Switch 62.5WG 14 oz	1, 2	5.1 a	12.6 a	42.7 a	39.4 ab	0.7 a	0.2 a	0.0 a	0.3 a
Luna Tranquility 27 fl oz	1, 2	4.0 a	18.6 a	43.5 a	33.5 ab	0.0 a	0.0 a	0.0 a	0.8 a
Miravis Prime 13.4 oz	1, 2	3.4 a	12.7 a	38.6 a	25.7 b	0.0 a	0.1 a	0.0 a	0.4 a

[z] Treatments were applied at (1) prior to first harvest and (2) after first harvest at each location. Application dates were as follows: 28 Apr and 14 May (Bacon SHB), 21 Apr and 13 May (Pierce SHB), 2 Jun and 11 Jun (Bacon Rabbiteye), and 26 May and 10 Jun (Pierce Rabbiteye).

[y] 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 Tukey's test (HSD)(α=0.05).

[x] Recorded for ~100-150 fruit collected on 17 May (Bacon SHB), 18 May (Pierce SHB), and 14 Jun (Bacon Rabbiteye and Pierce Rabbiteye).

[w] 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 Tukey's test (HSD)(α=0.05).

Evaluation of fruit rots after cold storage (field trial results). Fruit that was maintained in cold storage after harvest and periodically evaluated for rots did indicate fungicide treatment effects. In all cases, fruit harvested from plots where Miravis Prime or Luna Tranquility were applied showed significantly less rot development over time relative to the untreated control fruit (**Table 3**). In addition, in both the Bacon SHB and Pierce Rabbiteye sites, Switch applications also resulted in less fruit rot development over time. Treatments with Abound and Elevate did not significantly reduce rot development except in one location: the Pierce rabbiteye site.

Table 3. After cold storage rot evaluation results from all four field trial locations.

		Cold storage fruit rot development (AUDPC)[y]							
		All rots[x]				**Ripe Rot[w]**			
		SHB		**Rabbiteye**		**SHB**		**Rabbiteye**	
Treatment and amount/A	**Application timing[z]**	**Bacon**	**Pierce**	**Bacon**	**Pierce**	**Bacon**	**Pierce**	**Bacon**	**Pierce**
Untreated control	----	1456.7 a	n/a[v]	1007.7 a	1003.7 a	23.5 a	n/a[v]	0.0 a	0.0 a
Elevate 1.5 lb	1, 2	1267.8 ab	n/a[v]	894.4 ab	831.4 b	27.0 a	n/a[v]	0.0 a	0.0 a
Abound 15.5 fl oz	1, 2	1238.6 ab	n/a[v]	853.3 ab	788.6 bc	14.0 a	n/a[v]	0.0 a	0.0 a
Switch 62.5WG 14 oz	1, 2	1154.6 b	n/a[v]	764.6 ab	785.3 bc	8.8 a	n/a[v]	0.0 a	0.0 a
Luna Tranquility 27 fl oz	1, 2	1002.5 bc	n/a[v]	680.9 b	678.4 cd	13.9 a	n/a[v]	0.0 a	0.0 a
Miravis Prime 13.4 oz	1, 2	750.2 c	n/a[v]	687.8 b	616.1 d	2.2 a	n/a[v]	0.0 a	0.0 a

---

[z] Treatments were applied at (1) prior to first harvest and (2) after first harvest at each location. Application dates were as follows: 28 Apr and 14 May (Bacon SHB), 21 Apr and 13 May (Pierce SHB), 2 Jun and 11 Jun (Bacon Rabbiteye), and 26 May and 10 Jun (Pierce Rabbiteye).

[y] Rot development on cold storage fruit at intervals after harvest was used to calculate area under the disease progress curve (AUDPC) from ~100-150 fruit originally harvested on 17 May (Bacon SHB) and 14 Jun (Bacon and Pierce Rabbiteye). Rot was assessed at 9, 23, and 38 days after harvest (Bacon SHB), at 11 and 26 days after harvest (Bacon Rabbiteye), and 13 and 25 days after harvest (Pierce Rabbiteye).

[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 Tukey's test (HSD)(α=0.05).

[w] 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 Tukey's test (HSD)(α=0.05).

[v] Rot development in cold storage could not be recorded for the Pierce SHB site due to insufficient berries remaining after the grower mechanically harvested the trial plots prior to the trial harvest date.

Discussion

While the isolate identification portion of this work is ongoing, preliminary (partial) results suggest the presence of numerous fungal species on ripe blueberry fruit harvested in Georgia. Many of these species, especially *Colletotrichum sp.*, *Botrytis sp.*, and *Alternaria sp.* are well-known to cause in-field and post-harvest rots of blueberry, and control of these rots during the season using multiple fungicide applications is necessary in virtually all areas worldwide where blueberries are produced. Other species previously known to cause post-harvest fruit rots in blueberry were also identified in this study, including *Epicoccum sp.*, *Rhizopus sp.*, and *Aureobasidium pullulans*. Isolates from these species, as well as representative isolates from other fungal species identified in this work, will subsequently be assessed for their ability to cause post-harvest rots on ripe blueberry fruit under standard storage conditions. This work is ongoing as of the time of this report.

Based upon the results of the field trials, the impacts of fungicides applied near harvest (within 1-2 weeks prior to first harvest) appear to be minimal in terms of reducing rots (soft, leaky, sporulating fruit) at harvest. This is not completely unexpected. Many fruit rots are latent, and it is well-known that fungicide applications are most effective when used to prevent initial infection of blueberry fruit early in fruit development (such as during bloom and petal fall). Since the fruit examined in these trials were already formed, and turning blue, by the time the trial fungicides were applied, it is likely that many of these fruit were already infected prior to the initial trial fungicide application. Accordingly, each grower's spray program prior to the initiation of each respective trial likely had a more significant impact on observed rots than the two relatively late applications that were made as a part of these trials. In other words, these trial results reinforce the recommendation to use effective fungicides to control fruit rots early in the season (during bloom and petal fall) and suggest that a late ("rescue") fungicide application prior to harvest is not likely to make up for missed applications earlier in the season.

Nonetheless, despite the apparent lack of efficacy of these late fungicide applications at reducing rots at harvest, a significant reduction in post-harvest rot development in cold storage was noted on fruit harvested from trial plots treated with some fungicides in these trials. Specifically, Miravis Prime and Luna Tranquility applications reduced post-harvest rot development numerically and statistically in fruit harvested from all three assessed trial locations, and Switch applications reduced rot development in two of three locations. These results may be due either to residual antifungal activity of these products in harvested fruit or the ability of these products to clean up or reduce active fruit infections, thereby preventing subsequent infection of fruit after harvest within clamshells and storage containers. Since it takes time to sort, pack, and ship blueberry fruit prior to the fruit reaching market, these trial results suggest that fungicide applications prior to harvest may be of benefit in order to preserve fruit quality after harvest. Additional research would be needed to verify the efficacy and cost-effectiveness of in-field, preharvest fungicide applications for this purpose.

References

Bristow, P.R., Milholland, R.D., and Schilder, A.M.C. 2017. Botrytis Blight and Fruit Rot. Pages 17-19 in: Compendium of Blueberry, Cranberry, and Ligonberry Diseases and Pests, 2nd Edition. J. J. Polashock, F. L. Caruso, A. L. Averill, and A. C. Schilder. APS Press, St. Paul, MN.

Cooke, W.B. 1959. An ecological life history of *Aureobasidium pullulans* (de Bary) Arnaud. Mycopathol Mycol Appl. 12:1-45.

Mehra, L. K., MacLean, D. D., Savelle, A. T., and Scherm, H. 2013. Post-harvest disease development on southern highbush blueberry fruit in relation to berry flesh type and harvest method. Plant Dis. 97:213-221.

Milholland, R.D. and Cline, W.O. 2017. Alternaria Leaf Spot and Fruit Rot. Pages 9-10 in: Compendium of Blueberry, Cranberry, and Ligonberry Diseases and Pests, 2nd Edition. J.J. Polashock, F. L. Caruso, A. L. Averill, and A. C. Schilder. APS Press, St. Paul, MN.

Milholland, R.D. and Schilder, A.M.C. 2017. Phomopsis Twig Blight and Fruit Rot. Pages 34-37 in: Compendium of Blueberry, Cranberry, and Ligonberry Diseases and Pests, 2nd Edition. J.J. Polashock, F. L. Caruso, A. L. Averill, and A. C. Schilder. APS Press, St. Paul, MN.

Milholland, R.D. 2017. Anthracnose Fruit Rot. Pages 10-12 in: Compendium of Blueberry, Cranberry, and Ligonberry Diseases and Pests, 2nd Edition. J. J. Polashock, F. L. Caruso, A. L. Averill, and A. C. Schilder. APS Press, St. Paul, MN.

Wharton, P. and Schilder, A. 2015. Michigan Blueberry Facts: Fruit Rot Identification Guide. E2847. https://www.canr.msu.edu/resources/michigan_blueberry_facts_fruit_rot_identification_guide_e2847

White, T. J., Bruns, T., Lee, S., and Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: PCR Protocols: A Guide to Methods and Applications. M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White, eds. Academic Press Inc., New York.