Project Title: Understand the epidemiology of *Botrytis* to curb gray mold postharvest
WTFRC Project Number: CP-18-102

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Cooperators: Chelan Fruit, Stemilt

Report Type: Final Project Report

Project Duration: 3-Year

Total Project Request for Year 1 Funding: $32,360
Total Project Request for Year 2 Funding: $34,943
Total Project Request for Year 3 Funding: $33,371

Other related/associated funding sources

Agency Name: WSDA-Specialty Crop Block Grant program
Amt. awarded: $170,195
Notes: "Strategies to enhance pre- and postharvest management of gray mold in pome fruit" PI: Amiri, co-PI: Tobin Peever

WTFRC Collaborative Costs: None

Budget 1

Primary PI: Achour Amiri
Organization Name: Washington State University
Contract Administrator: Anastasia (Stacy) Mondy
Telephone: 509-335-2587
Contract Administrator Email Address: arcgrants@wsu.edu

Item	2018	2019	2020	2021
Salaries[1]	14,400	14,976	15,575	0
Benefits[1]	6,385	6,640	6,906	0
Wages[2]	5,760	5,990	6,230	0
Benefits[2]	545	567	590	0
Equipment	0	0	0	0
Supplies[3]	1,500	3,000	3,000	0
Travel[4]	1,070	1,070	1,070	0
Miscellaneous	0	0	0	0
Plot Fees[5]	2,700	2,700	0	0
Total	32,360	34,943	33,371	0

Footnotes:

[1] Salaries are for a Research Intern (0.3 FTE) at 44.3% benefit rate.

[2] Wages are for non-student temporary employee for summer help at 9.5% benefit rate.

3 Supplies include reagents for PCR and qPCR, agar media, plates and sampling materials.

[4] Travel to commercial and experimental orchards and packinghouses in WA for trials set -up, sampling and data collection.

[5] Plot fees for a block to be used for preharvest trial on gray mold in years 1 and 2.

OBJECTIVES:

Objective 1. Investigate infections timing of fruit by *Botrytis* in Washington apple orchard to determine critical timing of infections and subsequent disease expression in storage.

Objective 2. Evaluate the effect of preharvest weather conditions (rain and temperature) on infections and gray mold development in organic and conventional orchards.

Objective 3. Develop a timely preharvest spray management program to abate gray mold postharvest.

SIGNIFICANT FINDINGS:

- *Botrytis* was detected on flowers and fruit collected throughout the season from bloom to harvest.
- *Botrytis* was detected in the of orchard atmospheres throughout the season from bloom to harvest at low frequencies and variable among locations.
- The size of *Botrytis* inoculum was greater in organic orchards compared to conventional orchards.
- The inoculum size decreased from bloom to fruit set in conventional but then increased toward maturity and harvest. In organic orchards, the inoculum size increased throughout the season.
- Variabilities in inoculum size and dynamic throughout the season has been observed between orchards located in different districts.
- Effect of temperatures seems to be the factor that drives preharvest infection as summer infection tend to be lower as temperatures rise above 85°F.
- The impact of leaf wetness duration was hard to accurately assess across locations, but some microclimates and overhead cooling may increase infection risks.
- A spray program consisting of at least three sprays a season was effective in reducing gray mold development postharvest

RESULTS AND DISCUSSION

Objective 1. Infection timing of *Botrytis* preharvest and postharvest

As shown on Figure 1 below, *Botrytis* was detected in orchards at almost all sampling times. There seem to be a carry-over from bloom to fruit and increases as the fruit mature. Fungicide spray programs for each orchard were obtained and are being analyzed to correlate with potential fungicide effect on reduction of Botrytis load on fruit as this can be explained by the slight reduction observed before harvest (Figure 1) following the preharvest spray. However, the incidence of fruit infected (not decayed) with *Botrytis* increased significantly to 66% in Orondo and 58% in Mesa after 6 months of storage in CA. It is important to note that the fruits used in this study were not treated postharvest.

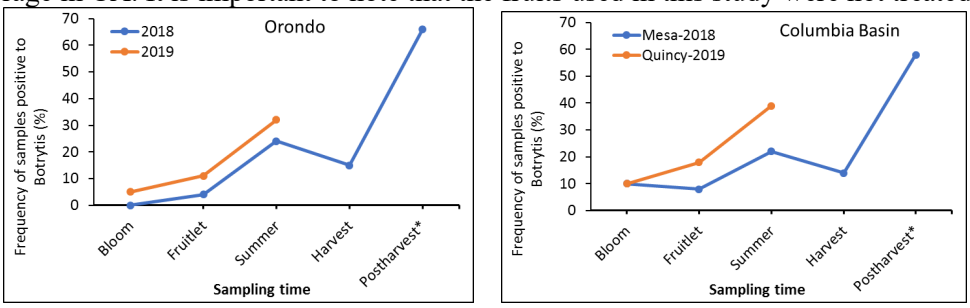

Figure 1. Evolution of *Botrytis* incidence throughout the preharvest growing season as detected by qPCR. 2019 Sampling after harvest are being analyzed.

Infections by *Botrytis* were observed in all parts of the fruit (cuticle, stem-end, calyx and inner flesh) at harvest at variable frequencies between orchards (Figure 2). This observation indicates that not only the external parts (calyx, cuticle and stem-end) of the fruit contains *Botrytis* inoculum at harvest, but also the flesh which indicates latent (dormant) infections from previous infections in the orchard. The frequency of samples carrying Botrytis remained steady or increased slightly in storage.

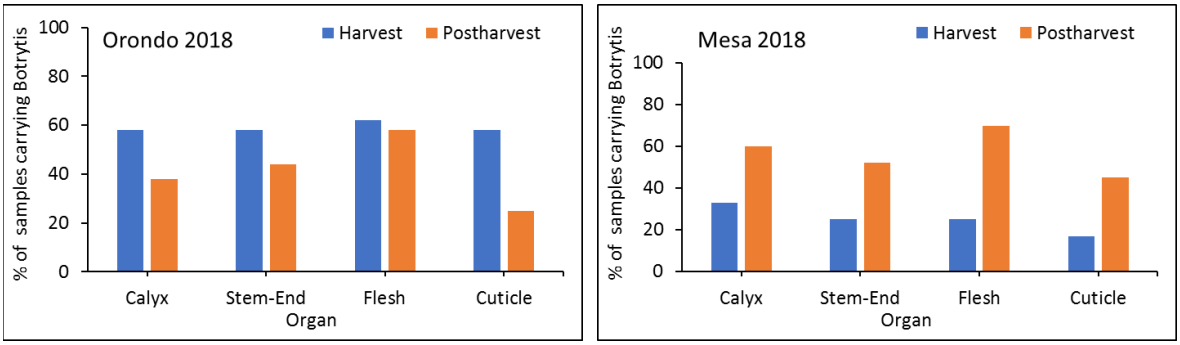

Figure 2. Incidence of *Botrytis cinerea* on different organs of the fruit at commercial maturity (harvest time) in organic and conventional orchards in 2018. Samples from 2019 Are being analyzed currently.

<u>Correlation between infection levels at harvest and gray mold incidence in storage:</u> There was a positive correlation between the incidence of Botrytis infections at harvest and gray mold incidence after 8 months of storage (Figure 3). Between 18 and 25% of fruit infected at harvest developed gray mold in storage but is was not proportional. Data of the 2020-21 season showed the same trend (data not shown).

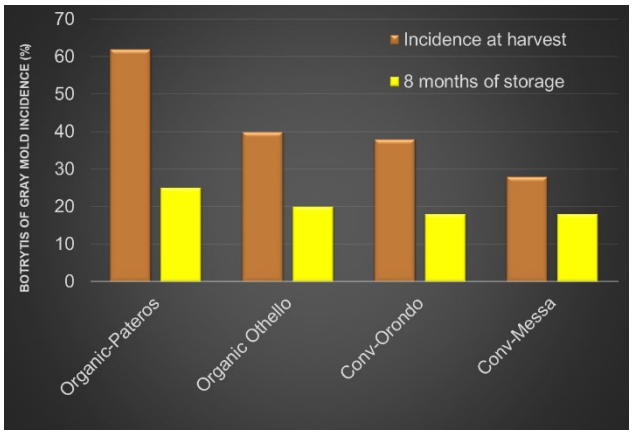

Figure 3. Botrytis incidence at harvest (brown bars) and gray mold incidence after 8 months if storage at 34°F in regular atmosphere on Fuji apples collected from four orchards in central Washington in 2019.

Objective 2. Impact of weather conditions on Botrytis incidence preharvest

Temperatures, rainfall, and leaf wetness duration (LWD) data have been collected from the experimental orchards for two growing seasons using the WSU AgWeatherNet platform. The weather stations were located within 2 miles radius from the experimental sites. Data were collected a week before and a week after our sampling to be able to make a close correlation. *Botrytis cinerea* infection risk is the highest at temperatures ranging from 68°F to 77°F and LWD of 8 to 14 hours. Our data showed variability between daily temperatures and LWD values between orchards (Figure 4). However, we see that daily average temperatures in 2019, were below 80°F except for the sampling done during the summer when temperatures were above 80°F. This would explain the slight decrease in Botrytis infection incidence that we saw during the summer across orchards (Figure 1). Except for the Mesa experimental orchard, LWD duration in 2019, were below 0.2 U (2 hours) during the periods between end of May and early September. The figure 3 clearly shows LWD values >6 hours for periods that correspond to our early (April-May) and late (preharvest-October) sampling when the infection by Botrytis was high. Temperatures were significantly higher in 2020 whereas LWDs values were slightly higher compared to 2019 (data not shown). In the 2020-21 season, more packers reported increased

gray mold incidence in cold storage. Whether this is weather-related only, will require more in-depth analyses of the weather data not just for one season but multiple seasons. The long-term objective behind this study is to make such clear correlation and develop potential risk models for Botrytis infections in the PNW.

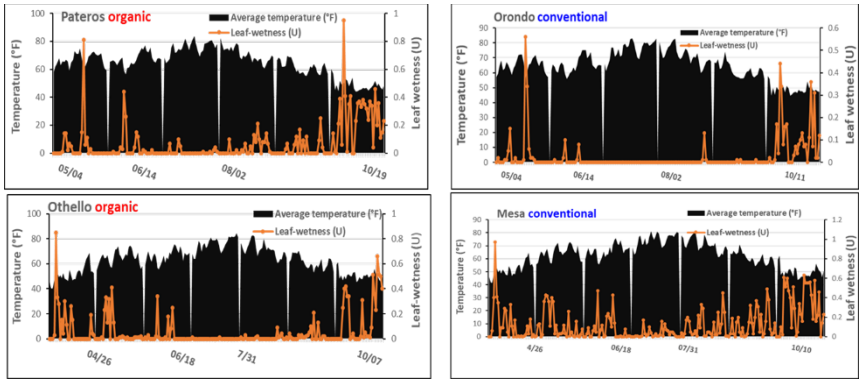

Figure 4. Temperatures (black lines) and leaf wetness duration (orange lines) values in four experimental orchards in 2019.

Objective 3. Develop a timely preharvest spray program to abate gray mold in storage

Spray programs consisting of Luna Sensation, Inspire Super, Fontelis, Topsin-M and Pristine were applied alone or in rotation duration two growing seasons on a Fuji block at the Sunrise orchard. Sprays were conducted at petal fall, fruit set (1-1.5" diameter), late-summer (August), and 7 days preharvest. We compared a conservative spray (1 spray/season), moderate-low (2 sprays), moderate (3 sprays) and extensive (4 sprays) to a non-treated control. Fuji apples were harvested at commercial maturity, stored at 35°F in regular atmosphere, and inspected for decay every 2 months. A summary of overall decay incidence and gray mold incidence are shown in Table 1. Our results indicate that fruit set spray combined with preharvest spray may be the best to reduce gray mold decay in storage. The one 7 day preharvest fungicide spray was not highly effective against gray mold compared to the other treatments. While a decision about the number of sprays a season may be economic, our future studies will aim at estimating the cost/benefits of moderate high and aggressive sprays versus conservative and moderate-low sprays considering cost of sprays and packout.

Table 1. Overall and gray mold decay incidence on Fuji apples sprayed with multiple fungicides at different phenological stages in 2019 and 2020.

		Decay incidence							
		Fungicide sprayed at				2019-20		2020-21	
Treatment type		Petall fall	Fruit Set	Late summ	7 DPH	Overall	Gray mold	Overall	Gray mold
	Untreated control	-	-	-	-	35	12	42	18
Conservative	1 spray-early	LS	-	-	-	11	5.0	13	6.0
	1 spray-early	-	LS	-	-	18	9.0	22	8.0
	1 spray-late	-	-	-	P	8.0	3.0	10	4.0
Moderate-Low	2 sprays-early	LS	IS	-	-	18	11	21	7.0
	2 sprays-mid/late	-	-	IS	P	7.0	3.0	9.0	3.0
Moderate-high	3 sprays-early/mid	LS	IS	F	-	8.0	3.0	10	2.0
	3 sprays-mid/late	-	F	IS	P	13	5.0	11	3.0
Aggressive	4 sprays	LS	TM	IS	P	6.0	1.0	8.0	1.5

LS= Luna Sensation, F = Fontelis, P = Pristine, IS = Insper Super, TM = Topsin-M

Executive summary

Project Title: Understand the epidemiology of *Botrytis* to curb gray mold postharvest
Key words: Gray mold, infection timing, weather, management

Abstract:

In statewide surveys conducted in 2016 and 2017 in Washington State, gray mold caused by *Botrytis* sp., was widespread and accounted for 30% to 40% of total decay on apple. Gray mold was found in 88% of grower lots surveyed across the state with incidences ranging from 5% to 75% of total decay across regions and lots. While Botrytis is known as a wet-region pathogen, it is surprising to see such relatively high gray mold incidences in the Pacific Northwest. This study aimed to define key infection timing of fruit by Botrytis, and we found an infection peak at petal fall and preharvest. Albeit infections seem to slow-down during the summer, they continue at a lower rate. We have also seen some effect of temperatures on Botrytis infections whereas the role of wetness in the regions still need future investigation. However, given the rate infection we saw at all sampling stage, indicate that *Botrytis* of pome fruit in Central Washington may be adjusting to shorter wetness periods if appropriate temperatures occur to infect fruit at a level that is enough to be challenging to packers in the region. Because of the semi-arid weather in central Washington, there may have been a tendence in the industry to wait until 14 to 0 days before harvest to make a spray. Our findings on key infection timing have been used to design and implement multiple spray programs at different phenological stages. Our data indicate that a program consisting of 3 sprays applied at fruit set, mid-summer, and 7 days preharvest (7 dph), reduces gray mold the best. The two first sprays may help in reducing latent infections that may develop early and mid-season while the last spay (7 dph) help reducing the newest infections that may occur closer to harvest when fruit are the most susceptible to infections.

OTHER OUTCOMES

Grants

Funds from this grant were leveraged to secure tow extra-mural grants, one from the Specialty Crop Block and another from the USDA-Crop Protection and Pest Management program to continue research on Botrytis epidemiology, role of weather conditions, fungicide resistance, *Botrytis* population genetics and pre- and postharvest management.

1. Epidemiology-based tactics to abate gray mold of pome fruit in the Pacific Northwest. USDA-NIFA CPPM. $199,805. P.I.: A. Amiri, Co-PI.: Karina Gallardo. 2020-2023.

2. Strategies to enhance pre- and postharvest management of gray mold in pome fruit. Specialty Crop Block Grant program (SCBG), WSDA-USDA. $230,155. P.I.: A. Amiri, Co-P.I.: T. Peever. 2019-2022

Abstracts and Manuscripts:

1. Amiri A., Mulvaney K.A., Pandit L.K., De Angelis R.D. **2017***. First report of resistance to fluxapyroxad and fluopyram in *Botrytis cinerea* from commercial apple orchards in Washington State. *Plant Disease* 101: 508.

2. Amiri A., Ali MD.E., De Angelis D.R., Mulvaney K.A., Pandit L.K. **2019***. Prevalence and distribution of *Penicillium expansum* and *Botrytis cinerea* in apple packinghouses across Washington State and their sensitivity to the postharvest fungicide-pyrimethanil. Proceedings of IV International symposium on Postharvest Pathology. Pp 1-4.

3. Amiri A., Acosta W. **2020**. Gray mold factsheet. http://treefruit.wsu.edu/crop-protection/disease-management/gray-mold/

4. Amiri et al. 2022. Determination of key infection timings of apple fruits by Botrytis cinerea in the Pacific Northwest. Plant Disease (Submitted)

5. Amiri et al. 2022. Optimization of preharvest spray programs in pome fruit orchards to abate gray mold decay in cold storage. Plant Disease (In preparation).

Talks

1. Wilson A., Amiri A. Management of gray mold pre and postharvest. *Northwest Apple Day*. Jan 21st, **2020**.

2. Amiri A. Management of Botrytis and Phacidiopycnis rots: field and storage. *Chelan Horticultural Meeting*. January 21st, **2019**.

3. Amiri, A. Epidemiology of Botrytis of pome fruit in the Pacific Northwest. *Western Pest and Disease Conference*, Portland, January 10th, **2019**

4. Bengyella L., Amiri A. Dynamic variation of Botrytis populations in pear orchards of the PNW. *WA Tree Fruit Association Annual meeting*, Yakima, December 4th, **2018**.