Project Title: Pre- and postharvest disease management in organic apple systems
WTFRC Project Number: CP-19-103A
Report Type: Final Project Report

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Project Duration: 3-Years/NCE

Total Project Request for Year 1 Funding: $67,715
Total Project Request for Year 2 Funding: $75,812
Total Project Request for Year 3 Funding: $80,991

Other funding sources: None

WTFRC Budget:

Item	2019	2020	2021
RCA Room Rental	6,300*	6,300	6,300
Total	6,300	6,300	6.300

Footnotes: RCA room(s) will be used to assess the efficacy of regular CA versus DCA for decay reduction.
*RCA rooms have not been used in 2019, so this amount will be used in 2020

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	2019	2020	2021	2022
Salaries[1]	38,400	39,936	41,533	0
Benefits[1]	14,008	14,569	15,151	0
Wages	0	0	0	0
Benefits	0	0	0	0
Equipment[2]	10,000	0	0	0
Supplies[3]	2,200	18,200	21,200	0
Travel[4]	1,007	1,007	1,007	0
Miscellaneous	0	0	0	0
Plot Fees[5]	2,100	2,100	2,100	0
Total	**67,715**	**75,812**	**80,991**	**0**

Footnotes:

[1] Salary for a PostDoc at $4,000/ month for 12 months at 0.8 FTE and benefit rate of 36.5%. The PostDoc will work jointly between Amiri' and Zhu's labs as needed.

[2] Funds for Safepod **or** Harvestwatch system for the DCA study in the present project and will be used in planned future research. We expect to obtain additional funds for one of these systems from the pending WSDA-SCBG grant if funded.

[3] Supplies include biological and microbiological reagents for fungi and fungicide tests, manuscript publication fees. In Year 2 and 3, we budget funds for molecular reagents and microbiome analyses work.

[4] Domestic travel to orchards in WA for trials, sampling and outreach activity.

[5] Annual plot fees for an experimental block at Sunrise to be used for the work outlined in the proposal below.

OBJECTIVES

1- Evaluate the adequacy and efficacy of current and novel preharvest management organic strategies.
2- Evaluate the benefits of using dynamic control atmosphere (DCA), GRAS products and biocontrol agents to control rots in storage.
3- Acquire novel knowledge about the impact of different spray regimes and storage conditions on fruit microbiomes pre- and postharvest to enhance management in the future.

SIGNIFICANT FINDINGS:

- ❖ The efficacy of 7 organic preharvest materials has been tested in 2019 and four to five of them show very good efficacy. In the 2020-21 season, 15 materials have bee tested preharvest. Efficacy of newer materials is seen, and consistent efficacy of the materials tested in 2019 is confirmed for most organic materials.
- ❖ The efficacy of these products was confirmed using artificial inoculations (Activity 1.2).
- ❖ Four most effective materials from 2019 trials were selected and tested in 2020 to develop a seasonal spray program (Activity 1.3) to enhance decay management. Overall decay incidence after 9 months of storage was positively proportional to the number of preharvest sprays.
- ❖ DCA and static CA (Activity 2.1) showed variability in reducing the incidence and severity of blue mold, gray mold, Mucor rot, Speck rot, and bull's eye rot. Trials conducted in the 2020-21 season confirm the slight advantage of DCA over CA or RA in reducing decays.
- ❖ Outreach: Some of the preliminary data have been provided to the stakeholders via meetings in 2020 and 2021.

Results and Discussion

Objective 1:

Activity 1.1. Efficacy of preharvest materials in reducing postharvest decays

<u>Season 2019-20</u>
Nine treatments, including a control (non-treated), a conventional fungicide (Merivon) and seven organic materials sprayed 7 days preharvest on a Fuji block in East Wenatchee in 2019.
After 8 months of storage at 34F in a regular atmosphere, overall decay (all decay types) incidence was 99% in the control and was reduced to below 50% by 5 organic products with Serenade Pro, Double Nickel and Regalia being the most effective (Figure 1). While OSO, provided a good efficacy against gray mold and blue mold when data were analyzed for each pathogen (Data not shown), it reduced overall decay incidence by 30%.

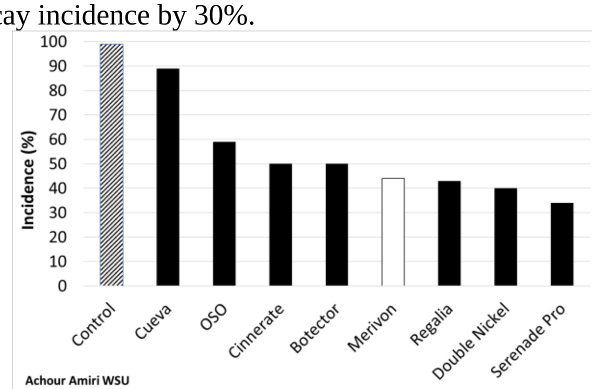

Figure 1. Overall decay (all decays combined) incidence on Fuji treated with the materials 7 days preharvest and stored at 34°F in a regular atmosphere for 8 months. <u>Season 2019-20</u>.

Season 2020-21

In 2020, 12 organic materials were sprayed 7 days preharvest and compared to the conventional Merivon and a non-treated control. The efficacy of most materials tested in 2019 was consistent and the 2020 trial showed that 8 organic materials significantly reduced overall decay incidence after 9 months of RA storage (Figure 2)

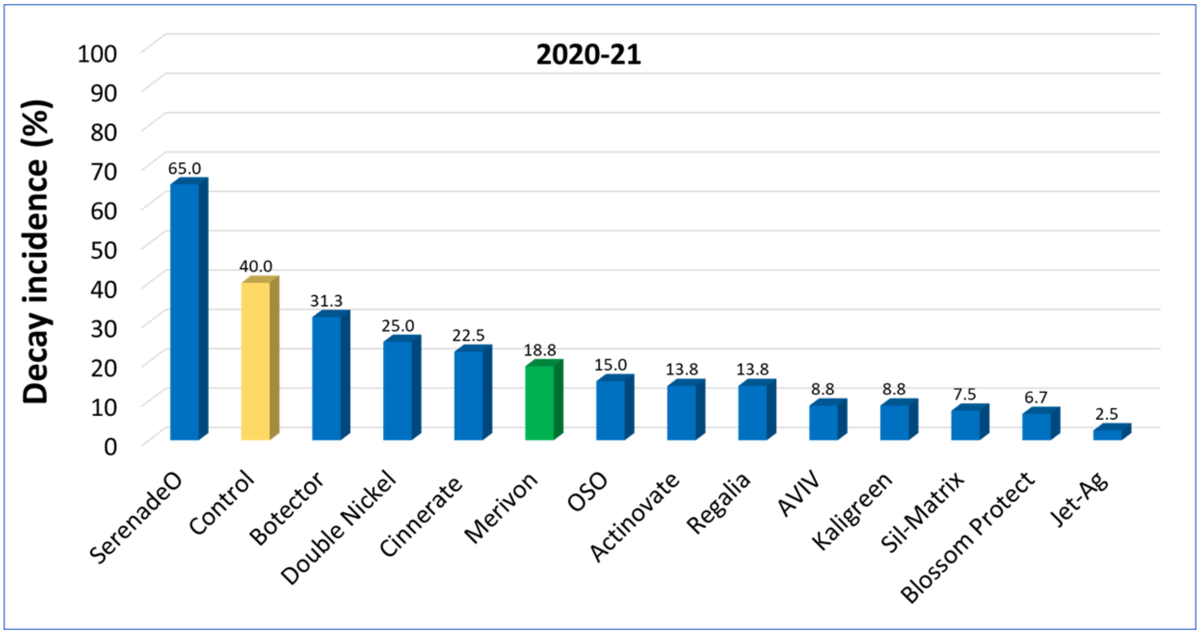

Figure 2. Overall decay (all decays combined) incidence on Fuji treated with the materials 7 days preharvest and stored at 34°F in a regular atmosphere for 8 months. Season 2020-21.

The trial was repeated during the 2021-22 season and data from the 3 or 2 seasons (Kaligreen, Cueva, Sil-Matrix, Jet-Ag, Actinovate) were averaged and shown in Figure 3.

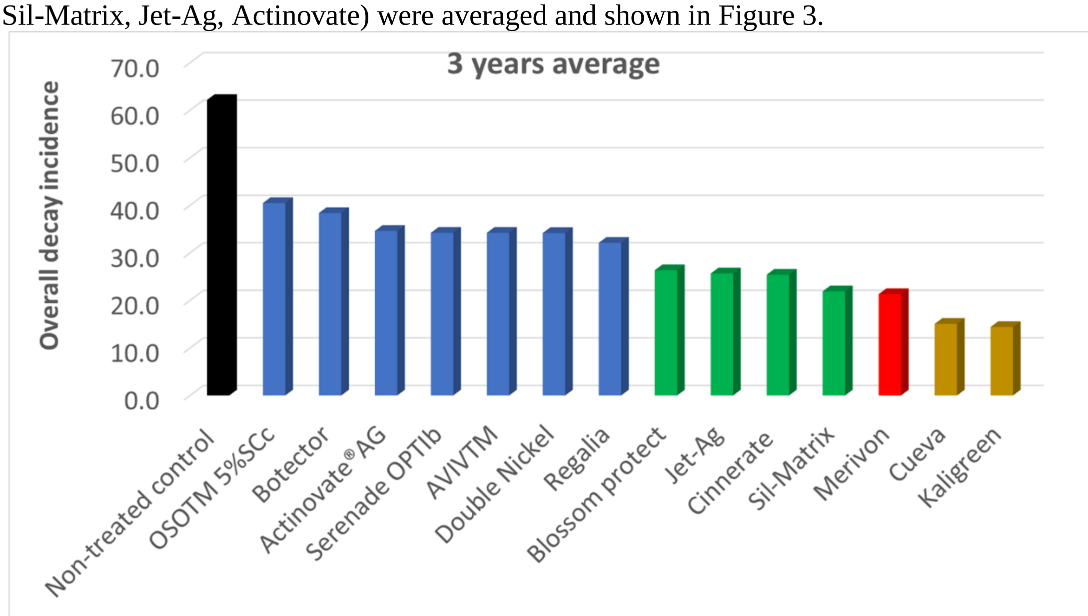

Figure 3. Average decay incidence (2 or 3 seasons) on Fuji treated with the materials 7 days preharvest and stored at 34°F in a regular atmosphere for 8 months.

Activity 1.3. Development of a timely preharvest organic spray program
Four organic materials which showed a good efficacy from Activity 1.1 were selected and sprayed sequentially from petal fall to 7 days preharvest. Results from the 2020-21 and 2021-22 seasons showed that decay incidence after 9 months of RA storage was better optimized with 3 to four sprays after petal fall especially for long term storage (Table 1). Some of the materials which showed a greater efficacy than the ones included in Table 1 have been tested in 2022-23 and data will be shared with stakeholders in 2023. We are also looking at overall spay programs that take into consideration other major preharvest diseases such as mildew and fire blight.

Table 1. Mid- and long term storage efficacies of different organic materials sprayed at different phenological stage.

# of	Spray conducted at				Storage term	
Sprays	Petal Fall	Fruitlet	Green Fruit	7 DPH	Mid-term (6 m)	Long-term (9 m)
3	OSO 5%	Cinnerate		Botector	100	100
3	Cinnerate	OSO 5%		Botector	100	90
4	Cinnerate	OSO 5%	AVIV	Botector	100	77
2	OSO 5%			Botector	91	41
3	OSO 5%		AVIV	Botector	90	64
1				AVIV	90	56
1				OSO 5%	90	27
1				Cinnerate	70	22
3	Cinnerate		AVIV	Botector	61	9
2	OSO 5%			AVIV	27	22
2	Cinnerate			AVIV	24	22
1				Botector	22	12
2	Cinnerate			Botector	-17	-72

Objective 2.

Activity 2.1. Efficacy of ultra-low oxygen (ULO) on decay development

Trials were conducted in 2020 and 2021 on Fuji artificially inoculated with spore suspensions of *P. expansum* (blue mold), B. cinerea (gray mold), *N. perennans* (bull's eye rot), *M. piriformis* (Mucor rot) and *P. washingtonensis* (in 2021, speck rot). Fruit were then stored in 3 different atmospheres, regular (RA), CA (O_2 = 4% and CO_2 = 0.8%), and ULO (O_2 = 1.5% and CO_2 = 0.8%) and stored at 35°F for 6 to 8 months. Results shows that ULO program tested in 2020r had a slight reduction of incidence of blue mold, gray mold, and bull's eye rot but not mucor (Figure 4). In 2021, DCA showed similar efficacy with greater reduction seen for speck rot and bull's eye rot.

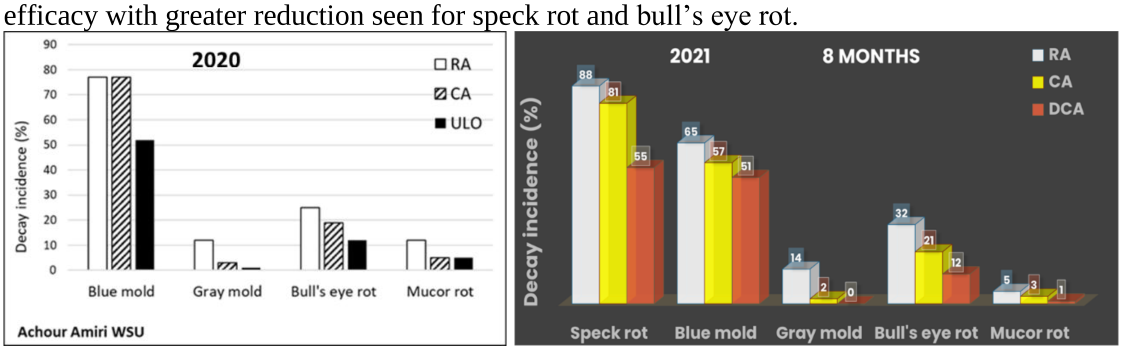

Figure 4. Average decay incidence of 5 major postharvest diseases on artificially inoculated Fuji stored in 3 different atmospheres for 6 months in 2020 and 8 months in 2021.

Objective 3. Acquire novel knowledge about the impact of different spray regimes and storage conditions on fruit microbiomes pre- and postharvest to enhance management in the future.

Apples from the cultivars Gala and Honeycrisp were collected from four orchards, i.e., one conventional and one organic orchard, for each cultivar in 2021 (Gala) and 2022 (Honeycrisp). Twenty Gala apples were harvested at commercial maturity from orchards located near Brewster, WA. Three samples, i.e., peel, calyx-end and stem bowl, were obtained from each fruit. DNA was extracted from the samples and microbiome analyses using 16 S rRDNA (bacteria) and ITS3 x ITS 4 (fungi). Samples from Gala from Washington State were compared to Gala samples from 8 other countries (Figure 5). Gala from Washington state seem to harbor less fungal species compared to Gala from other European countries (Figure 5). Interestingly, organic and conventional Gala apples from WA were not significantly different in term of fungal diversity although the organic apples had a slightly greater diversity.

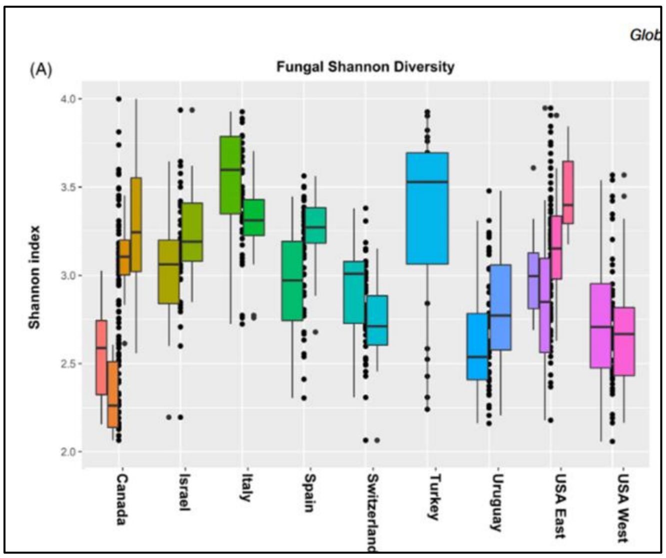

Figure 5. Box plots showing the fungal diversity (Shanon index) of apple samples from WA (right pink bars) in comparison with 7 other countries.

The fungal population of WA Gala apples seems to be the closer from that of Italian Gala than Gala from the US-east coast (Figure 6 b), whereas the bacterial population of WA Gala seem to be distinct from those of East-coast Gala and the 7 other countries surveyed (Figure 6d)

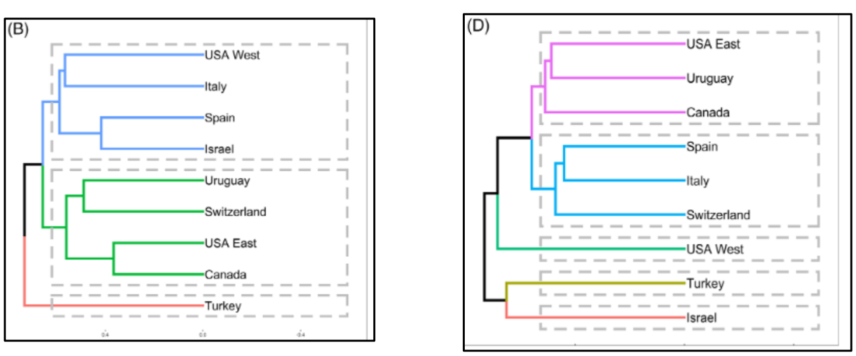

Figure 6. Dendrogram of hierarchal clustering showing the similarity between fungal (B) and bacterial communities of Gala apples from different countries.

Analysis of fungal and bacterial communities from Gala apples form a conventional orchard (B) and an organic orchard in WA, showed strong similarities between tissue types from two different systems with the peel harboring more diverse fungal and bacterial communities and organic apples usually having a slightly more diverse populations but not significantly different or conventional apples (Figure 7).

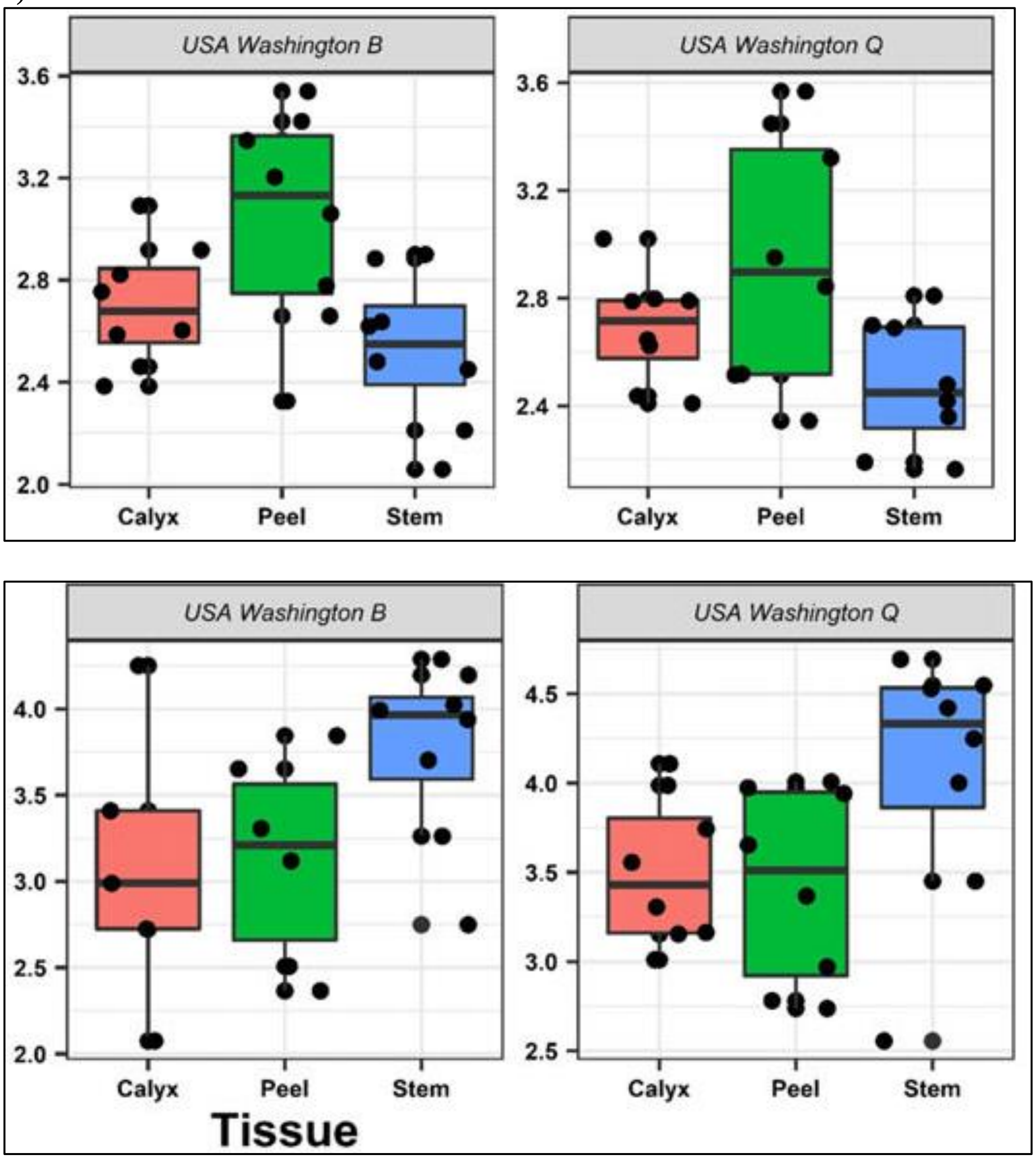

Figure 7. Box plots showing the fungal diversity (top) and bacterial diversity (bottom) among apple tissues, Calyx, peel, stem, of Gala apples collected from a conventional (USA Washington B) and an organic (USA Washington Q) in Washington State in 2020.

Samples (flowers and fruit) from Honeycrisp were obtained from one conventional and one organic orchard in Quincy in 2021. Beside flowers collected at full bloom, fruit were collected at green stage (July), at commercial maturity (harvest), and after 3 months of storage in a regular atmosphere. For fruit, 4 sample types, i.e., cuticle, flesh, stem-end, calyx-end were obtained. DNA was extracted and stored at -80°C (-121°F). Samples are awaiting microbiome analysis which was delayed due to our initial collaborator taking a different position. Results are expected in 2023 and will be shared through extension meeting in 2023 and Workshop in 2024.

Executive Summary

Project title: Pre- and postharvest disease management in organic apple systems

Key words: Decays, preharvest, postharvest, organic management, DCA

Abstract: Herein, we report the efficacy of most current organic material commonly used by organic growers preharvest against major postharvest decays. Fourteen organic materials were screened for 3 consecutive seasons in the field and on detached fruit using artificial inoculations. Materials included biologicals, plant extracts, salts and bio-fungicides. Our results show the ones that are expected to provide a good level of efficacy up to 8 months in storage when applied prior to harvest. The variability in efficacy seen in some cases for the same product between seasons indicate potential inconsistencies known, especially, for biologicals. This also warrants alternation of different materials throughout the season to optimize control in storage. We have also compared different spray programs at different phenological stages to optimize postharvest management. One spray, 7 to 0 day preharvest may be enough for short term storage (up to 4-5 months) but may not protect fruit longer. We found that sprays at petal fall and fruit set, using different materials, in addition to a preharvest spray can significantly reduce decay incidence for up to 8 months. To optimize decay control in organic systems further, we assess the efficacy of a dynamic controlled atmosphere in reducing decays. While the approach still requires some fine-tuning across cultivars, DCA can reduce the development of some latent pathogens such as *B. cinerea* (gray mold), *P. washingtonensis* (Speck rot), and *Neofabraea spp.* (bull's eye rot) better than standard static CA. Based on the results from Gala collected from two orchards in the same location (Brewster), organic and conventional apples from WA do not seem to differ significantly in term of overall bacterial and fungal populations they harbor. While the results need to be discussed in more details in relation to postharvest d pathogens, they could indicate that the last preharvest treatment applied just before harvest may not have significantly impacted the fungal and bacterial populations in the short period of before fruit processing.

Additional Items

Grants

Funds from this grant were leveraged to secure tow extra-mural grants, one from the Organic Research Extension Initiative (OREI) and another from the Western SARE Program to further continue research and extension efforts to support organic growers and packers in Washington.

1. Pre and postharvest disease management of pome fruit to support an expanding organic production in the Pacific Northwest. USDA-Western SARE. $349,887. P.I.: A. Amiri, Co-PIs. K. Gallardo (04/22-08/25).
2. A systems-based approach to enhance quality, safety, and shelf life of organic tree fruit in the Pacific Northwest. USDA-NIFA-OREI. $1,499,887. P.I.: A. Amiri, Co-PI.: C. Torres, F. Critzer, K. Gallardo, B. Sallato (07/21-04/25)

This is equivalent to $8 brought for each $1 invested by the WTFRC in this project.

Abstracts and Talks:

1. Amiri A. 2023. Organic Disease Management: Challenges and Opportunities. OPDMC, Portland, OR. Jan 12th 2023.

2. Amiri A. and Fomba J. 2022. *In vitro* activity of several organic materials against the major postharvest pathogens of Pome fruit. Annual Meeting of the American Society of Phytopathology. Pittsburg, PA. August 2022.
3. Amiri A & Fomba J. 2022. Update on efficacy of organic materials to minimize decay in storage. WA Tree Fruit Association Annual meeting. Dec 6^{th}, 2022.
4. Amiri el. 2022. Webinar on organic decay management: provided 4 talks related to organic decay management. Attendees: 90.
5. Amiri A. 2021. Update on efficacy of organic materials and research needs to minimize decay in Honeycrisp. WA Tree Fruit Association Annual meeting. Dec 7^{th}, 2021.