FINAL PROJECT REPORT
WTFRC Project Number: AP-16-105

Project Title: Improved risk assessment and management of apple postharvest diseases

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Cooperators: Multiple packers in Washington. Syngenta, Decco, Pace.

Other funding sources: None

WTFRC Collaborative Expenses: None

Total Project Request: **Year 1**: $67,121 **Year 2**: $67,534 **Year 3**: $67,635

Budget 1
Organization Name: WSU **Contract Administrator:** Katy Roberts/Kim Rains
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Item	**2016**	**2017**	**2018**
Salaries[1]	39,600	41,184	42,831
Benefits	15,721	16,350	17,004
Wages	0	0	0
Benefits	0	0	0
Equipment[2]	2,000	0	0
Supplies[3]	8,000	8,000	6,000
Travel[4]	1,800	2,000	1,800
Miscellaneous	0	0	0
Plot Fees	0	0	0
Total	67,121	**67,534**	67,635

Footnotes:
[1] Salaries are for Postdoc (1.0 FTE) at 39.7% benefit rate.
[2] Equipment will include costs for an Air-Sampler to monitor the airborne fungal population.
[3] Include costs for lab supplies i.e. sampling tubes, microbiological media and plates for fungal growth and fungicide sensitivity tests.
[4] Travel to packinghouses and orchards.

OBJECTIVES

1. Conduct a multiyear statewide decay survey program to detect and quantify decay risks.
2. Evaluate risks related to fungicide resistance
    a. Develop rapid and accurate methods for fungicide sensitivity evaluation.
    b. Conduct a multiyear statewide resistance monitoring program on fruit and in storage room atmospheres.
3. Evaluate the impact of storage conditions on resistance development in the blue and gray mold pathogens in relation to fungicide type.
4. Evaluate sensitivity/resistance to pre-harvest fungicides i.e. newly registered ones and non-target sprays in major pome fruit pathogens.
5. Evaluate pathogenicity and fungicide sensitivity of *Lambertella* and *Phacidium* rots, newly reported in pome fruit in WA.

SIGNIFICANT FINDINGS

Objective 1: *Conduct a multiyear statewide decay survey program to detect and quantify decay risks*

- ❖ 325 grower lots were surveyed across all apple growing regions in central Washington between January and June, 2016 and 2017. Blue and gray molds were predominant and accounted for 48 and 25% of total decay, respectively.
- ❖ The “export” quarantine pathogens were found at about 10% of total decay with Bull’s eye rot being the most predominant one in this group. Speck rot (Phacidiopycnis) was at about 1% whereas Sphaeropsis was found very sporadically.
- ❖ The newly reported Lambertella rot, now known as Yellow rot was found in 34% of lots surveyed with an incidence ranging from 2 and 40% per grower lots. The newly reported pathogen, Phacidium rot, was not found sporadically.

Objective 2: *Evaluate risks related to fungicide resistance*

2-b- Conduct a multiyear statewide resistance monitoring program on fruit and in storage room atmospheres

- ❖ Nearly 6,000 isolates of *Penicillium expansum* (blue mold) and 4000 isolates of *Botrytis cinerea* (gray mold) were collected from different packinghouses in 2016 an d2017. These isolates were tested for sensitivity to 6 fungicides: thiabendazole (Mertect), pyrimethanil (Penbotec) and fludioxonil (Scholar) for both *P. expansum* and *B. cinerea* and to pyraclostrobin + boscalid (Pristine) and fluxapyroxad (Merivon) for *B. cinerea* only.
- ❖ Resistance of *P. expansum* to thiabendazole (Mertect) and pyrimethanil (Penbotec) was found in about 60% and 52% of the 325 lots surveyed, respectively.
- ❖ Resistance of *B. cinerea* to pyrimethanil, TBZ, Pristine, and fluxapyroxad (Merivon) was found in 55%, 46%, 38% and 15% of the 325 lots surveyed, respectively.
- ❖ Populations of *B. cinerea* and *P. expansum* with reduced sensitivity to fludioxonil were found in several packinghouses. These populations are controlled by the label rate of the fungicide. However, continuous use of Scholar and related products can cause these populations to become actually resistant.

- ❖ A total of 325 decay and resistance profiles were shared with the participating packers and growers before the beginning of the new season to allow them change strategies and spray regimes based on decays and resistance found at their locations.

Objectives 3:

- ❖ Genes known to increase the risk for fungicide resistance development in *B. cinerea* seem to be up-regulated (increased expression) by cold temperatures usually used to store apples which may increased the risk of selecting for resistance especially for Penbotec and Scholar.
- ❖ For *P. expansum*, the expression of some genes is lowered at cold temperatures while other genes could be up-regulated.
- ❖ Further Research is needed to better assess the risk and find solutions that may help with reducing the expression of the genes involved.

Objectives 4:

- ❖ The efficacy of Academy (fludioxonil + difenoconazole) was evaluated against 10 major apple pathogens in vitro (lab) and on fruit
- ❖ Preliminary results indicate that Academy would be effective against blue mold, gray mold, yellow rot, Speck rot ad moderately effective against Mucor.
- ❖ A second-year study is going on to further confirm the efficacy levels seen in 2017-18 season.
- ❖ Currently, Academy is only available for drench application and the waste management process for difenoconazole has limited the use of this new fungicide in commercial packinghouses. If a formulation for a dry application is developed (expectation for the 2020 season), Academy can be a valuable tool to add to the few existing fungicides.

Objective 5: *Evaluate pathogenicity and fungicide sensitivity of Lambertella and Phacidium rots, newly reported in pome fruit in WA.*

- ❖ Nine major apple cultivars were tested for susceptibility to *Lambertella corni-maris* and *Phacidium lacerum*. All cultivars were infected when inoculated throughout wounds but some cultivars such as Honeycrisp, Fuji, Piñata and Gala were more susceptible.
- ❖ Fludioxonil (Scholar) and pyrimethanil (Penbotec) controlled isolates of *L. corni-maris* on detached apple fruit whereas TBZ failed to provide any control. The preharvest fungicide Pristine provided only a moderate efficacy (30 to 50%).

RESULTS AND DISCUSSION

Objective 1. *Postharvest diseases prevalence*

Blue and gray molds accounted for almost 72% of total decay observed with blue mold being predominant with 48% of total decay (Figure 1-left). Blue mold was detected in 157 of the 160 lots surveyed versus 132 lots for gray mold. A majority of lots had less than 20% incidence of gray mold, whereas a higher number of lots had between 40 and 80% blue mold (Figure 1-right). Besides these two mains decays, bull's eye rot was found in 52 lots at frequencies ranging from 1 to 75%, whereas the statewide frequency was 4.3%. The frequency of the "crabapple diseases" Speck rot and Sphaeropsis rot was 2.5 and 1.4%, respectively. It is possible that better management practices, including pruning and appropriate fungicide sprays, resulted in such low frequencies compared to those reported when these two pathogens were first described in the state. Additional minor pathogens included Alternaria rot (2.9%) and the newly reported yellow rot (2%). Other minor or non-identified decays accounted for 14.3% of total decay.

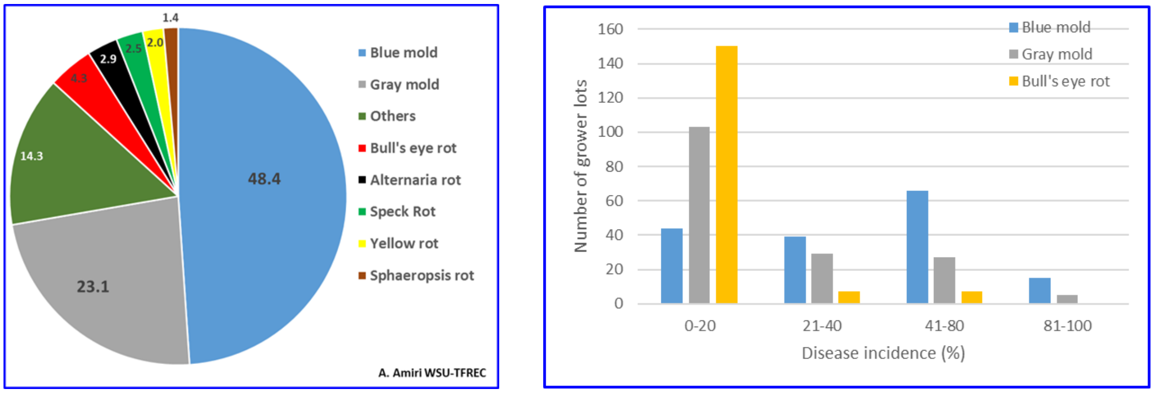

Figure 1. Overall incidence of major postharvest diseases found in Washington in 2016 (left) and incidence distribution of blue mold, gray mold and Bull's eye rot among grower lots (Right).

Objective 2. *Fungicide resistance occurrence and frequencies*

Over the 2,000 of *Penicillium expansum* (blue mold) isolates tested, 24% and 16% were resistant to TBZ and pyrimethanil (Penbotec), respectively. About 11% had reduced sensitivity to fludioxonil (Figure 2-left). Over the 1,700 of *Botrytis cinerea* (gray mold) isolates tested, 14% and 20% were resistant to TBZ and pyrimethanil (Penbotec), respectively, whereas 12% had reduced sensitivity to fludioxonil (Figure 2-right). Overall, 11% and 3% of *Botrytis* isolates were resistant to the pre-harvest fungicides Pristine and Merivon.

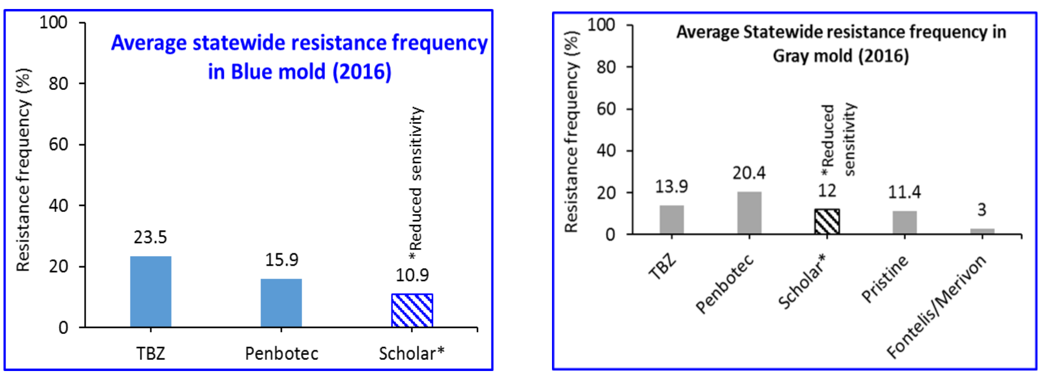

Figure 2. Overall resistance frequencies to major pre- and postharvest fungicides in blue mold (left) and gray mold (right) observed statewide in 2016.

In blue mold, about 66% of grower lots showed resistance to TBZ and more than 30% had a resistance frequency >40% (Figure 3, left). For pyrimethanil 55% of lots showed resistance with a largest portion having between 1 and 20% resistance. Interestingly, about 45% of lots surveyed showed reduced sensitivity to fludioxonil (Figure 3, left). Resistance was slightly lower in gray mold, compared to blue mold, with the highest frequency observed to pyrimethanil (Figure 2, right). In a non-negligible portion of lots surveyed, the same fungicide was used for more than one year which may explain their highest resistance frequencies compared to state average.

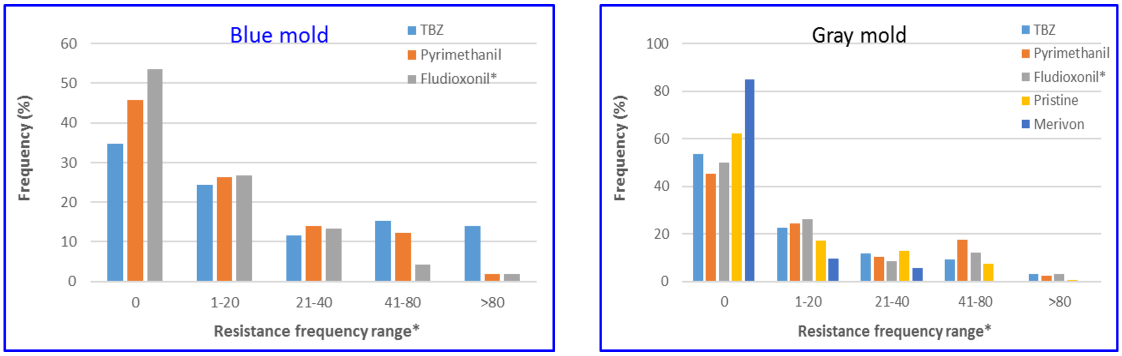

Figure 3. Resistance frequency distribution in blue mold (left) and gray mold (right) observed statewide in 2016. * for fludioxonil, it is only reduced sensitivity.

Objective 3: *Evaluate the impact of storage conditions on resistance development in the blue and gray mold pathogens in relation to fungicide type*

In pome fruit, fungicides are applied at harvest and fungicide residue are constant (do not degrade) over a long storage period at 33°F to 36°F. Therefore, if the fungus is present on the fruit/bin or in the room, the contact between the fungicide and the fungus is continuous which may increase the selection pressure and cause more resistance to occur. We evaluated the expression of genes known to be involved in fungicide resistance in both *B. cinerea* and *P. expansum* at 34°F (1°C) and 36°F (20°C) over a 6-month period. Our preliminary results show that the expression of the studied genes seems to be increased at low temperature when using pyrimethanil (PYR) or fludioxonil (FDL) especially in resistance isolates. It seems also that the low temperature alone, without the fungicide, may cause an increase in the expression of the genes especially in *Botrytis*. More experiments are ongoing to better understand the risks and look at other potential genes and results will be presented at the Apple Review Day in January 2019.

Objective 4: *Evaluate sensitivity/resistance to pre-harvest fungicides i.e. newly registered ones and non-target sprays in major pome fruit pathogens*

Trials were conducted in the 2016-17 season to evaluate the efficacy of Difenoconazole and Academy against major postharvest diseases. The same trial has been reconducted in the 2017-18 season. Difenoconazole (alone) was applied at harvest on fruit artificially inoculated before harvest and after harvest (for Lambertella, Penicillium and Mucor only). Results on Figure 4 indicate that after 4 months of storage, most of preharvest fungicides are controlled, whereas Lambertella and Penicillium were significantly reduced compared to the control. Incidence of Mucor rot was reduced from 80% in the control to 50% in the difenoconazole-treated fruit.

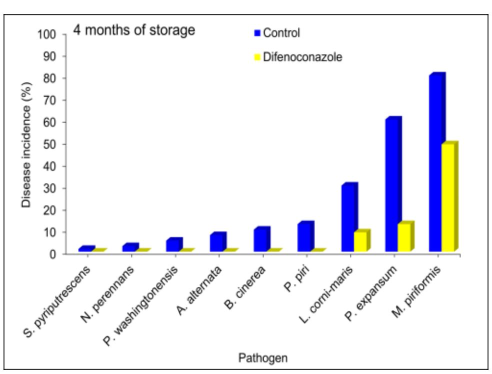

Figure 4. Efficacy of Difenoconazole against 9 pathogens

Objective 5: *Prevalence of yellow and Phacidium rots and their sensitivity to pre- and postharvest fungicides*

Yellow rot (Lambertella) was found in 34% of lots surveyed with an incidence ranging from 2 and 40% per grower lot. Overall, 96% of the lots surveyed had an incidence of 2 to 10% whereas only 4% showed a yellow rot incidence higher than 10%. Phacidium rot was very sporadic and seldom found in both years of survey.

The susceptibility of 9 apple cultivars to yellow rot (*L. corni-maris*) shown in Figure 4 indicates that Red Delicious is the least susceptible cultivar together with Cameo and Granny Smith being significantly less susceptible than the remaining cultivars. On the other hand, Honeycrisp and Gala are among the most susceptible ones (Figure 5).

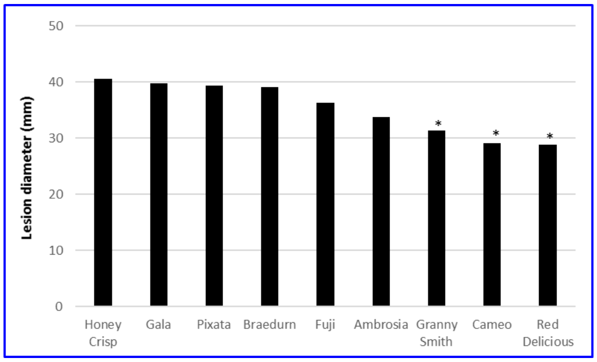

Figure 5. Susceptibility (expressed as lesion diameter) of most common apple cultivars to yellow rot (Amiri et al. Plant Disease, 2017). * indicate cultivars significantly less susceptible.

Yellow rot is totally controlled by fludioxonil at label rate while pyrimethanil provided a high efficacy (>94% control) on fruit wounded and inoculated with the fungus (Figure 6). To the contrary, TBZ failed to provide any efficacy against yellow rot. This is not due to fungicide resistance but rather to inherent inefficacy of this group of fungicide against yellow rot. It is not clear yet if *L. corni-maris* infect fruit pre- or postharvest, however the preharvest fungicides Topsin-M (same group as TBZ) and Pristine (pyraclostrobin + boscalid) may not provide adequate control if fruit are infected in the orchard.

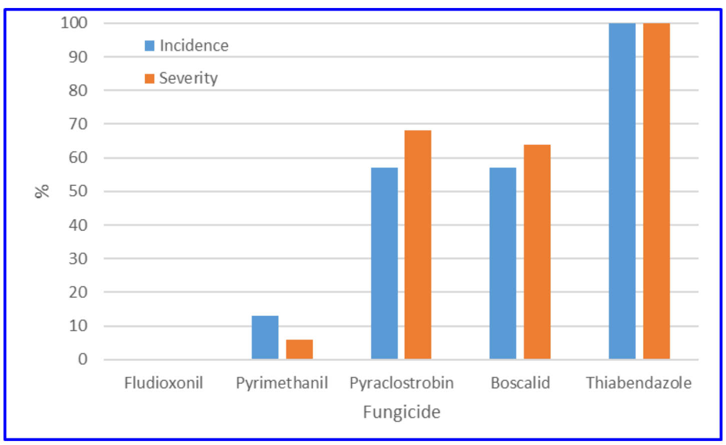

Figure 6. Efficacy of pre- and postharvest fungicides against yellow rot on apple fruit (Amiri et al. Plant Disease, 2017).

Executive summary

The long-term known apple diseases in addition to the emerging new pathogens, mostly reported from Washington, have put a tremendous pressure on growers and packers in recent years in term of improving management and reducing losses. We planned for a first step consisting of knowing the exact and real risks and their extent to develop sustainable solutions. We conducted two years of statewide decay surveys combined with a statewide fungicide resistance monitoring to assess any possible impact in recent shifts in cultural and management practices and climate change on emergence or exacerbation of pathogen populations or increased resistance frequencies. Such information was highly needed to improve fruit production sustainability. Fungicide resistance monitoring programs will be crucial to pinpoint location-specific problems, evaluate the potential impact of environmental conditions between regions and different spray regimes on resistance development. Moreover, the impact of target and no-target sprays in orchards as well as storage conditions on decay control efficacy and potential resistance development problems need to be assessed to improve disease management.

Summary of findings: We have a better understanding of the risks caused by apple pathogens in term of occurrence, distribution and importance. More specifically, we acquired new knowledge about the emerging quarantine pathogens (*Lambertella, Phacidiopycnis, Lambertella, Phacidium*) in term of importance, control and susceptibility of major cultivars. One the major outcomes from the project is a better assessment of existing risks of fungicide resistance in pome fruit systems in Washington. Although, resistance has emerged to most fungicides, levels of resistance can be considered lower compared to other crop systems. However, caution is needed to avoid catastrophic scenarios due to control failure is resistance continue to increase because rationale solution and practices are not implemented. We have also evaluated the efficacy of the newest postharvest fungicide, difenoconazole, against major diseases which will make it a useful tool in the future due of its efficacy and different mode of action suitable for fungicide resistance management.

Project Outcomes:

- Peer reviewed publications: 5 (3 more pending)
- Extension publications: 5
- Professional presentations: 8
- Extension presentations: 25

Future Directions:

- Develop specific management programs for major disease: Blue mold, gray mold and Bull's eye rot.
- Better understand the epidemiology of preharvest pathogens to enhance management in storage.
- Continue research efforts in assessing the effect of storage conditions on decay and fungicide resistance development and develop solutions to them.
- Acquire more knowledge about the emerging (quarantine) pathogens in term of epidemiology and best management practices.