FINAL PROJECT REPORT

Project Title: Epidemiology and management of postharvest decay on pears

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Total Project Funding: $90,737

Budget History:

Item	2017-18	2018-19
WTFRC expenses	0	0
Salaries Faculty Research Assistant	22,500	23,175
Benefits OPE 63%	14,198	14,624
Wages	0	0
Benefits	0	0
Equipment	0	0
Supplies	6,000	6,180
Travel	2,000	2,060
Miscellaneous	0	0
Plot Fees	0	0
Total	$44,698	$46,039

Footnotes: Annually: FRA 6 mo + fringe, 6K supplies and consumables, 2K local and in-state travel, 3% inflation

ORIGINAL OBJECTIVES

1. Monitoring prevalence of major fungal pathogens throughout the pear growing season towards understanding postharvest disease epidemiology

   Major goal of this objective is to identify the time period in growing season where the prevalence of major fungal pathogens is highest. Monitoring at preharvest conditions allows us to determine the sources of inoculum, patterns of spread, and modes of entry to the fruit tissues. The information generated from this objective will help in developing preharvest spray schedules and/or assessing the risk of postharvest infection and thus following subsequent disease management strategy.

2. In vitro sensitivity of postharvest decay pathogens to currently available fungicides and efficacy of new fungicides toward resistance management

   Major goal of this objective is to quickly assess the efficacy of currently available fungicides as well as the population dynamics of major postharvest pathogens in terms of fungicide resistance. Since fungicides have been the major control strategy for most of the postharvest pathogens, the tendency of developing resistance is equally high. In vitro screening of the available fungicides to major fungal pathogens would allow us to determine the resistance and/or efficacy of certain group of fungicides and thus direct the identification of new fungicides and management of fungicide resistance.

3. Manipulating postharvest storage conditions to reduce the susceptibility of fruit infection

   The major goal of this objective is to identify the best storage conditions to minimize the postharvest decay and thus enhance the longevity of pear storage. Different storage conditions and treatments that minimize the infection by major fungal diseases will be identified. The information generated from this will be combined to the pre/postharvest fungicide application to develop a sustainable disease management strategy to combat postharvest decay in pears.

SIGNIFICANT FINDINGS

- Postharvest rot pathogens, *Botrytis cinerea, Cladosporium herbarum, Penicillium expansum,* and *Alternaria* spp. were prevalent in orchards at the initial stages of fruit development
- Three new pathogens, *Dothiorella omnivora*, *Diaporthe rudis,* and *Fusarium* sp. were isolated from bloom time samples and occasionally isolated from culled samples in packing houses (not included in this study)
- *Botrytis cinerea* causing gray mold on postharvest fruits were isolated most frequently during bloom time and in lower frequencies after petal fall and field bins.
- Some isolates of *B. cinerea* tested in this study are resistant to the fungicides used for scab management; triflumizole, cyprodinil, and dodine that may result in indirect selection of resistant *B. cinerea* population to these groups of fungicides
- One hundred percent of isolates tested in this study were sensitive to a group of fungicide, iprodione (FRAC 2) that is not registered for pear in PNW
- Preharvest application of 1-MCP (Harvista) did not result in fungicidal properties against *Botrytis cinerea.* However, the storage capability of fruits under normal atmosphere increased by reducing senescence damage on Harvista applied fruits two weeks prior to harvest.

- The negative effects of 1-MCP on fruits ripening after storage may be mitigated by hanging fruits longer after 1-MCP application pre-harvest.

RESULTS & DISCUSSION

Objective 1: Monitoring prevalence of major fungal pathogens throughout the pear growing season towards understanding postharvest disease epidemiology

Based on culture and spores morphology, four pathogens previously reported as postharvest rot pathogens, *Botrytis cinerea, Penicliilum expansum, Caldosporium herbarum,* and *Alternaria* spp. were identified. These pathogens were consistently isolated from full white, full bloom, petal fall, fruitlets, and field bins at harvest. Several fungal species were isolated along with the pathogenic species, the identity of which could not be established based on culture and spore morphology. They were grouped into thirteen unique species based on cultural characteristic on PDA media. The molecular diagnostic methods using the ITS and elongation factor 1-alpha (EF1-α) sequencing, thirteen unique fungi were identified. The thirteen species were tested in surface sterilized wound inoculated fruits for their ability to cause disease. Out of thirteen, three species were identified as pathogenic on wound inoculated fruits (Figure 1, continuing project report 2019, page 101). The three new species were identified as *Dothiorella omnivora, Diaporthe rudis, and Fusarium* sp. A plant disease note on first report of *D. rudis* causing fruit rot of European pears in the United States is published in Plant Disease journal (https://doi.org/10.1094/PDIS-12-18-2184-PDN). Interestingly, the two species *D. omnivora* and *D. rudis,* also cause trunk disease in grapes (a complex of many fungal pathogens). With the increasing wine grape acreage in the area, we might not only be sharing the acreage but also the pathogens to some extent. It will be important to keep monitoring for newer pathogens as the area's commercial agriculture change over time.

This study shows that these pathogens can colonize the floral organs at early stages of fruit development, can follow through picking bins, and possibly end up to the storage facilities (Figure 2, continuing project report 2019, page 101). We recovered highest percentage of *B. cinerea* isolates from full bloom and petal fall stages and lowest from the fruits at picking bins during commercial harvest. *Botrytis cinerea* can cause both calyx and stem end gray mold in storage. Calyx end gray mold is generally initiated by infection of calyxes in the orchard close to full bloom, remaining latent throughout the growing season and resulting calyx end gray mold in storage. The stem end gray mold can initiate from cracks or damage at stem end during harvest or postharvest handling during transport and storage. Once the infection starts, the decay spreads in the storage within a container to the nearby fruits. These results suggest for integrating management approaches of gray mold storage rot between seasonal management in orchard and post-harvest management at packinghouses.

Several *Alternaria* spp. were recovered from early stages of fruit development to picking bins. The species were collectively recorded as *Alternaria* spp. in this study as the sequencing of ITS only did not delineate the species complex. The genus *Alternaria* includes more than 280 species that can be both pathogenic and saprophytic. On pears and apples, *Alternaria alternata*, *A. tenuissima, A. arborescens*, *A. ventricosa, and A. yaliinficiens* are reported as pathogenic species causing Alternaria rot, pear black spot, core browning, and moldy core. The isolated *Alternaria* spp. from this study will be further characterized for their species diversity and pathogenicity on four cultivars of European pears including Bartlett, Green Anjou, Comice, and Bosc.

Penicillium expansum and *Cladosporium herbarum* were recovered in similar frequencies until petal fall stages. At fruitlet and picking bins the recovery percentage of *P. expansum* stayed similar as earlier stages, however the recovery percentage of *C. herbarum* increased significantly at these stages with *C. herbarun* being the frequently recovered species at picking bins. At fruitlet and picking bins stages, the tissues used for isolation included calyx end and stem end tissues. At these stages, *Alternaria* spp., *B. cinerea, C. herbarum, and P. expansum* were the only pathogenic species recovered from the

samples. Both *P. expansum* and *B. cinerea* are the most common pathogens causing storage rot and can initiate in calyx end, stem end, or wounded sites on fruit surface. Species like *Alternaria* spp., and *C. herbarum* on the other hand are weak pathogens and infect compromised fruits such as wounds or senescing fruits. Recovery of these species at two major infection sites during harvest suggests the possibility of inoculum being carried to the storage for both calyx end, and stem end rot, as well as for the undiscovered wound sites during postharvest handling of fruits.

The three new pathogens, *Diaporthe rudis, Dothiorella omnivora,* and *Fusarium* sp. were recovered at lower percentages until petal fall. At fruitlets and picking bins, they were not recovered. However, these pathogens were occasionally isolated from post-harvest fruits with storage rot symptoms in packinghouses. Given the type of tissues used at these two stages in this study, there may exist other conduits for pathogen inoculum to initiate storage rots in packinghouse.

Objective 2: In vitro sensitivity of postharvest decay pathogens to currently available fungicides and efficacy of new fungicides toward resistance management

The effective concentration to reduce radial growth by 50% (EC_{50}) was calculated for each pathogen-fungicide combination. The EC_{50} for isolates against cyprodinil ranged from 1.35 to 26 µg ml^{-1}. No discriminatory dose for use on pears was found in the literature, but a 1 µg ml^{-1} was considered a discriminatory dose for *B. cinerea* on New Zealand wine grapes (Beresford et al 2017), and all isolates except one exceeded this limit by at least threefold. The range of EC_{50} values against dodine was 73.4 to 195.7 µg ml^{-1}. No baseline or discriminatory dose information for *B. cinerea* was found in the literature. While the EC_{50} values seem high, the labeled rate for the product used to supply this active ingredient is also high, with a maximum of 1,485 µg active ingredient ml^{-1}. The range of EC_{50} values against iprodione was 0.53 to 1.32 µg ml^{-1} No discriminatory dose for use on pears was found in the literature, but all isolates were below the 10 µg ml^{-1} concentration determined in a study of *B. cinerea* on Southern US strawberries (Fernández-Ortuño et al 2014). The range of EC_{50} values against triflumizole was 0.38 to 1.53 µg ml^{-1}. Except for cyprodinil, the tested isolates were sensitive within labeled field rates. Two isolates (10%) were not sensitive against cyprodinil at the maximum labeled field rates and EC_{50} for 85% isolates were greater than 5 µg/ ml.

Among, the isolates tested for fungicide efficacy, 100% of the tested isolates were sensitive to iprodione, a fungicide group that is not registered for pear in PNW (Figure 3 and 4, continuing project report 2019, page 102). Only 30% of the isolates were sensitive to triflumizole and all the sensitive isolates were collected from SOREC research orchards. The remaining of other isolates (70% of the tested isolates) with reduced sensitivity to triflumizole were collected from conventionally managed commercial orchards. Triflumizole (FRAC group 3) includes a common group of fungicides, including Inspire Super for managing pear scab diseases and powdery mildew. Among the tested isolates, 65% and 75% of the isolates were sensitive to dodine and cyprodinil respectively. Even though dodine is currently not used for commercial application, it was a fungicide of choice for scab management until the resistance became an issue. Similarly, cyprodinil (FRAC group 9) is also an important component of pear scab management. While the resistance frequencies to these groups among the tested isolates are relatively low precautionary measures should be taken for fungicide rotations. Identification of a group of fungicide with high efficacy, iprodione (FRAC group 2) is one of the major accomplishments of this project. However, the results need to be verified with larger number of isolates and further steps needs to be taken toward registration and labeling.

Objective 3: Manipulating postharvest storage conditions to reduce the susceptibility of fruit infection

2017-Preharvest 1-MCP application and wound inoculated fruits: Preharvest application of foliar 1-MCP alone did not significantly reduce the wound initiated *B. cinerea* infection in cold storage for both bosc and comice pears. The disease progress over time was lower on Bosc fruits treated with 1-MCP a week prior to harvest at minimum rate; however, it was not statistically significant. Disease progress on other treatments were significantly higher than water control treatments. Similar result was observed on comice fruits treated with 1-MCP. Application of 1-MCP at preharvest is not fungicidal enough to control the disease caused by wound initiated *B. cinerea.*

2017-Preharvest 1-MCP application and normal atmosphere stored fruits: The fruits stored in normal atmosphere cold storage from 2017 pre harvest 1-MCP application resulted comparable firmness relative to control treatments. At two months after storage, Harvista applied comice fruits two weeks prior to harvest were firmer than fruits with Harvista treatments one week prior to harvest and control treatments with no Harvista application. However, all fruits were below 2.5 lbs after five days of ripening. At four months after storage, no significant differences were observed on any of the Harvista treated fruits. All fruits were below 1.5 lbs after five days of ripening (Fig. 5). Similar results were observed on bosc fruits (Fig. 6). At two months after storage, Harvista treated fruits were firmer compared to no harvista treated control fruits. At four months after storage, no significant differences were observed among the treatments. At six months after storage, fruit texture data were difficult to interpret due to hardening of outer layer. That could be due to loss of moisture resulting rubbery texture of fruit periderm and subsequent difficulties for the probe to puncture fruits for texture data. Differences in percent senescence were observed among the treatments. In bosc after six months of storage, the percent senescence on the fruits treated with Harvista two weeks prior to harvest resulted in zero senesced fruits with minimum rate and 35% senesced fruits with maximum. However, on fruits treated a week before harvest resulted in 50 and 55% senesced fruits with minimum and maximum rate. The fruits with no Harvista treatment resulted 80% senesced fruits (Fig. 7)

The results were promising from this study as the firmness/ripeness of the fruits were not affected by pre harvest application of 1-MCP and that the fruits can be stored longer with less loss to senescence. However, these data was generated from only one years of study. We repeated the two weeks prior to harvest application with two rates in 2018 and other objectives were added for the treatments effect.

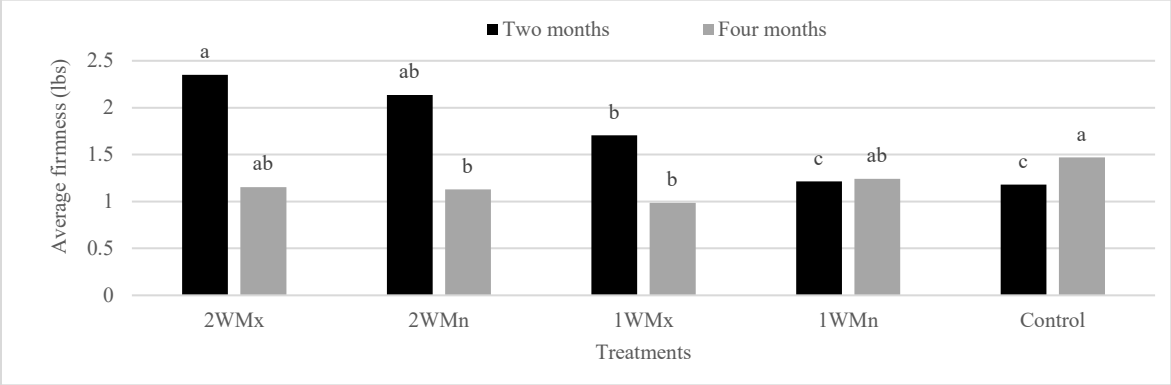

Figure 5: Post-storage fruit texture on Harvista applied **comice**. The bars with same letters are not significantly different ($P<0.05$*)*

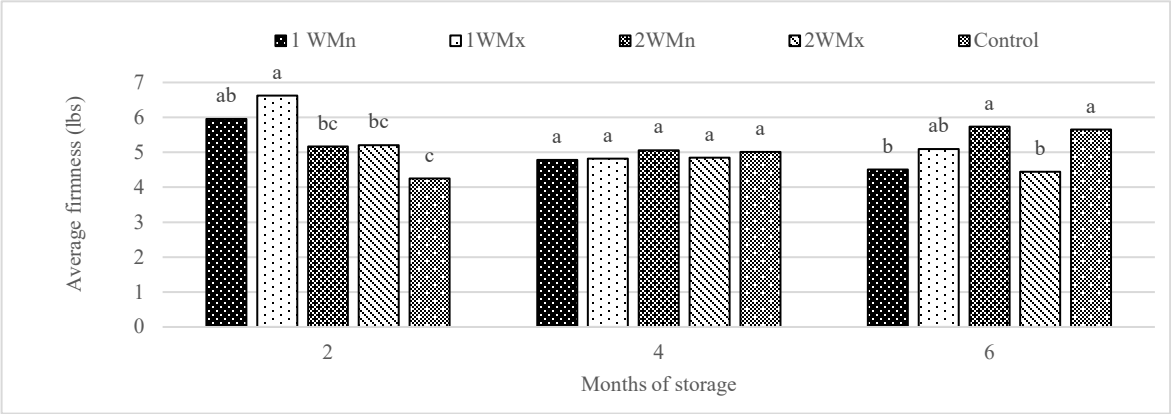

Figure 6: Post-storage fruit texture on Harvista applied **bosc**. The bars with same letters are not significantly different (*P*<0.05*)*

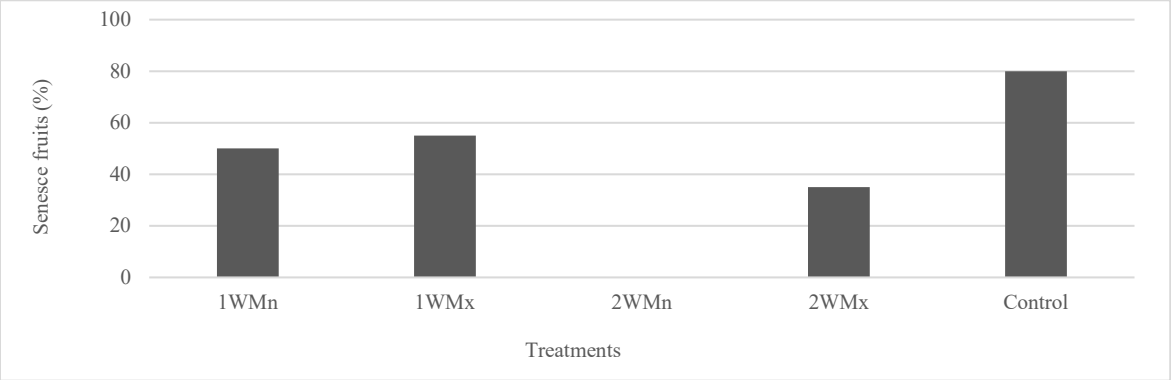

Figure 7: Percent senescence on **bosc** stored in normal atmosphere cold storage for six months.

2018-Preharvest 1-MCP application and normal atmosphere stored fruits: We picked fruits at three different time points (commercial harvest, a week, and two weeks after commercial harvest) and analyzed the efficacy of 1-MCP to maintain fruit texture in tree for better harvest planning. Bosc fruits at commercial harvest were picked two weeks after Harvista application, however due to the sudden drop in fruit texture in comice fruits in 2018, the commercial harvest fruits were picked three days after Harvista application. The average fruit texture on Bosc at the day of Harvista application was 16 lbf. The texture ranged from 14 to 15 lbf two weeks after application; and 13 to 14 lbf three and four weeks after application. The fruit texture on Bosc treated with maximum rate of 1-MCP were consistently one unit firmer compared to minimum rate and control fruits in all harvest time points. No significant differences on fruit texture were observed between fruits treated with minimum rate of 1-MCP and the non–treated control fruits. The fruits treated with maximum rate of 1-MCP dropped two units from the day of application to 21 days later, whereas the ones with minimum rate of 1-MCP and control dropped three units (Fig. 8a). This effect was not significant in comice pears. Due to early harvest in comice, the final harvest commenced 17 days after Harvista application. Although no significant differences on fruit texture were observed 17 days after application among the treatments, the fruits treated with 1-MCP tended to stay firmer than control fruits in all harvest time points. The average fruit texture on comice at the day of Harvista application was 11.9 lbf. The texture ranged from 10.7 to 11.8 lbf three days after application; 10 to 10.9 ten days after application; and 9.9 to 10.3 lbf seventeen days after application (Fig. 8b). Besides maintaining fruit texture, one of

the benefits of leaving fruits longer on trees would be fruit size. However, leaving fruits on trees two weeks longer than commercial harvest did not make significant differences on fruit weight in Bosc and increased by less than an ounce in comice (Fig. 8c, Fig. 8d).

The another set of harvested fruits were stored in both normal atmosphere cold storage and controlled atmosphere cold storage. Fruits were stored in cardboard boxes in 2018 compared to plastic bins in 2017 to protect fruits from moisture loss during long-term storage. Bosc and comice fruits were stored for six and four months respectively in normal atmosphere cold storage and five and four months respectively in controlled atmosphere cold storage. After storage, the fruits were allowed to ripen at room temperature for seven days. The fruits firmness were measured and the data on fruit texture were analyzed. Similar to the preharvest fruit texture on Bosc, the fruits treated with maximum rate of 1-MCP were approximately one unit firmer than control fruits. Within the maximum rate of 1-MCP treated fruits, the fruits got softer when picked a week and two weeks after commercial harvest. The average post-storage fruit texture on maximum 1-MCP treated fruits picked at commercial harvest was 9.1 lbf that reduced to 7.7 lbf on fruits picked one and two weeks after commercial harvest (Fig. 9a). On comice, no significant differences were observed between the treatments. However, the fruit texture decreased significantly on fruits harvested one week and two weeks after commercial harvest including maximum 1-MCP treated fruits (Fig. 9b). Similar results were observed on fruits stored at controlled atmosphere cold storage (Fig. 9c, Fig. 9d). These results suggest the negative effects of 1-MCP on fruits ripening after storage may be mitigated by hanging the fruits longer after 1-MCP application pre-harvest. However, this study needs to be validated with more years of data and with additional cultivars.

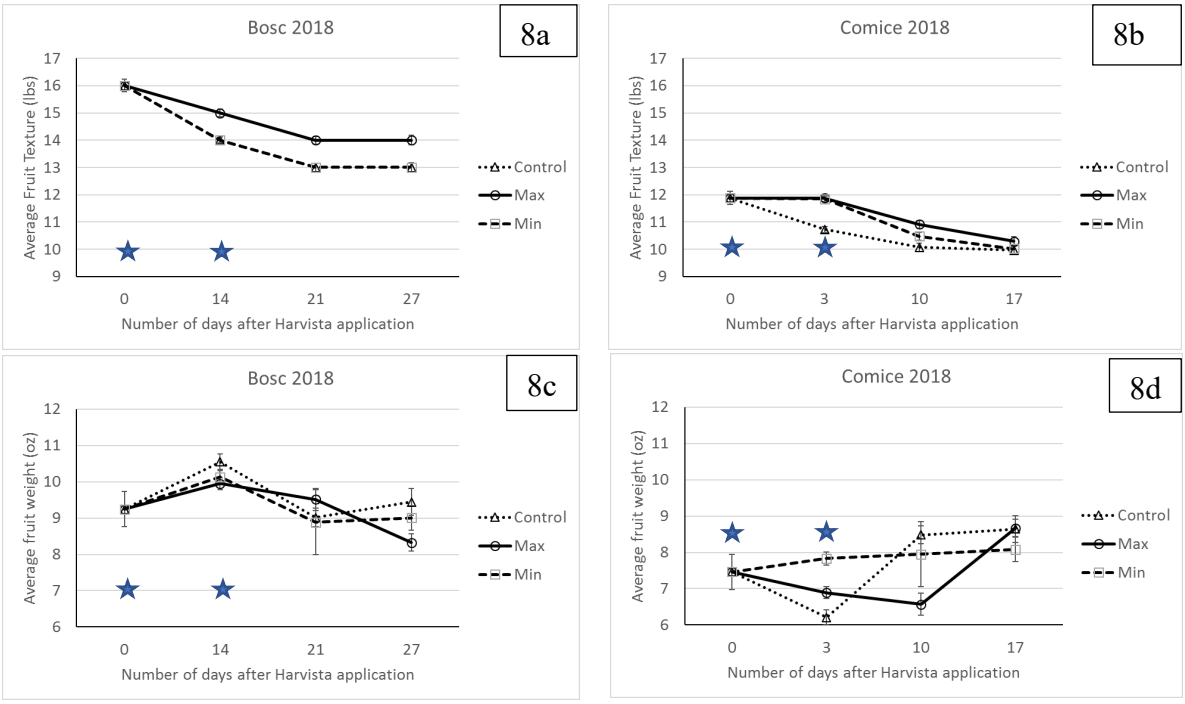

Figure 8: Pre-storage fruit texture (a & b) and fruit weight (c & d) on Bosc and Comice. The data represent an average of 40 fruits. The star symbols represent day of Harvista application and commercial harvest days on respective cultivars.

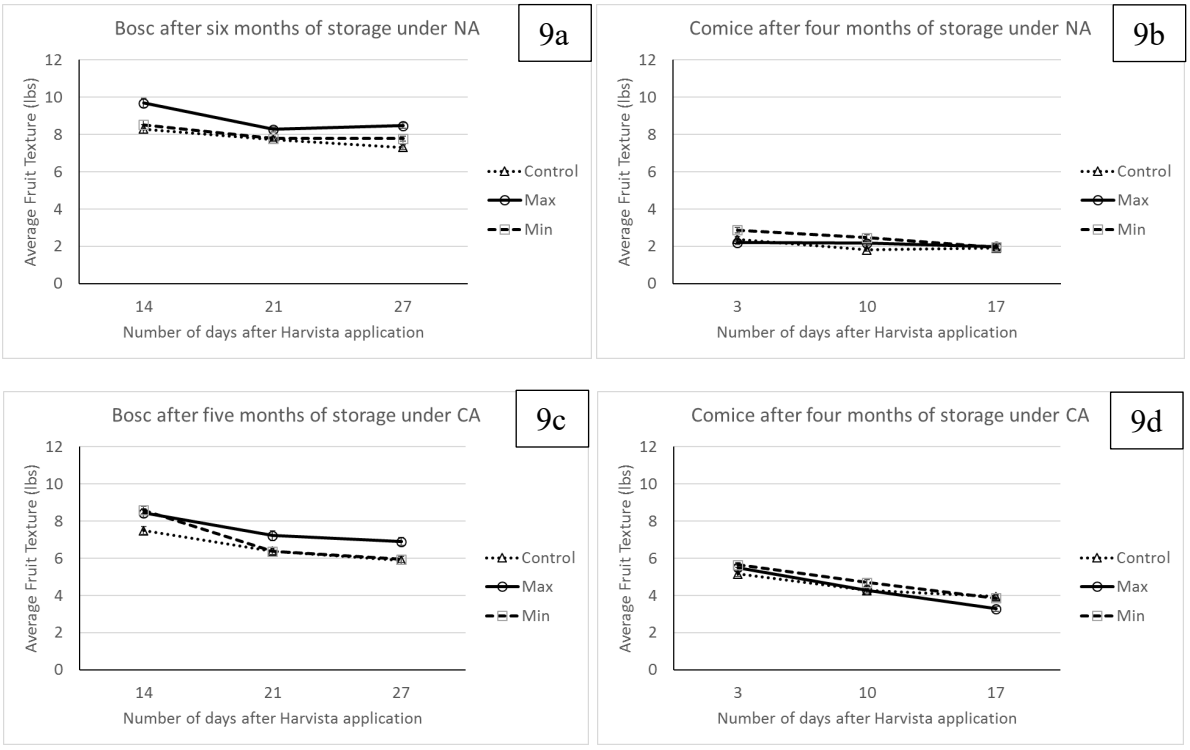

Figure 9: Post-storage fruit texture on Harvista applied bosc and comice stored under natural atmosphere (a & b) and controlled atmosphere (c & d). The data represent an average of 40 fruits per treatment. The fruits were picked 14, 21, and 27 days after Harvista application, where 14 days is the commercial harvest date.

EXECUTIVE SUMMARY

Project title: Epidemiology and management of postharvest decay on pears

Key words: postharvest decay, fungicide sensitivity, 1-MCP

Abstract: Postharvest decay in pears is caused by several fungal pathogens. This study identified several of these pathogens colonizing tissues from initial stages of fruit development, and fungicides and other products that can be applied at bloom time and integrated into existing pre-harvest and postharvest fungicide management programs.

Summary of findings:

- Postharvest rot pathogens, *Botrytis cinerea, Cladosporium herbarum, Penicillium expansum,* and *Alternaria* spp. were prevalent in orchards at the initial stages of fruit development
- Three new pathogens, *Dothiorella omnivora*, *Diaporthe rudis,* and *Fusarium* sp. were isolated from bloom time samples and occasionally isolated from culled samples in packing houses (not included in this study)
- *Botrytis cinerea* causing gray mold on postharvest fruits were isolated most frequently during bloom time and in lower frequencies after petal fall and field bins.
- Some isolates of *B. cinerea* tested in this study are resistant to the fungicides used for scab management; triflumizole, cyprodinil, and dodine that may result in indirect selection of resistant *B. cinerea* population to these groups of fungicides
- One hundred percent of isolates tested in this study were sensitive to a group of fungicide, iprodione (FRAC 2) that is not registered for pear in PNW
- Preharvest application of 1-MCP (Harvista) did not result in fungicidal properties against *Botrytis cinerea.* However, the storage capability of fruits under normal atmosphere increased by reducing senescence damage on Harvista applied fruits.
- The negative effects of 1-MCP on fruits ripening after storage may be mitigated by hanging the fruits longer after 1-MCP application pre-harvest.

Future directions:

Continuing monitoring of newer pathogens as the commercial agriculture dynamics of Southern Oregon changes: Most pathogens share several hosts and it is possible that either the newer pathogens for storage rots are introduced or the existing pathogens get severe due to the availability of alternate hosts.

Developing an integrated fungicide management programs that will include bloom time, pre-harvest, and postharvest applications: Given the frequencies of pathogens isolated at different stages, an integrated approach to target stage specific pathogens appears necessary for managing different kinds of storage rot. At the same time fungicides with different modes of action needs to be evaluated for their efficacy against these pathogens as well as continued monitoring of individual pathogen for their shift in sensitivity to commonly used fungicides.

Continued testing of 1-MCP application as pre-harvest treatment: As we were narrowing the optimum treatments (rate and timing of application, timing of harvest) for pre-harvest 1-MCP applications, the results are produced from one year of field applications. The results look promising but the repeatability of the treatments and results need to be validated with additional sites, cultivars, and years.