FINAL PROJECT REPORT

Project Title: Effect of crabapple pruning on Sphaeropsis and speck rot incidence

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Other funding sources: None

Total Project Funding: $63,191

WTFRC Collaborative Expenses:

Item	2013	2014	2015
Salaries			
Benefits			
Wages[1]	137	137	
Benefits	66	66	
RCA Room Rental[2]	2,100	2,100	
Shipping			
Supplies			
Travel[3]	190	190	
Plot Fees			
Miscellaneous			
Total	$2,493	$2,493	

Footnotes: [1]Wages and Benefits @ $15/hour (two trees pruned per hour); [2] Storage for approximately 50 cartons of fruit (1/3 of a RCA room@ $6,300 per room); [3]Travel @ $0.555 per mile for two trips each to 3 sites in central Washington.

Budget History:
Organization Name: Pace International

Item	2013	2014	2015
Salaries			
Benefits			
Wages[1]	8,640	12,980	8,986
Benefits[2]	2,851	4,283	2,965
Equipment			
Supplies[3]	4,500	4,500	2,500
Travel[4]	2,500	2,500	1,000
Plot Fees			
Miscellaneous			
Total	$18,491	$24,263	$15,451

Footnotes: [1] Wages for a part-time research technician to work 720 hrs in year 1 and 3 and 1040 hrs in year 2 at $12/hr for performing sample collection, pathogen isolation, decay evaluation, and data management. The increase in wages for years two and three reflects a 4% rate increase. [2] Benefits 33%. [3]Supplies include culture media, chemicals, petri-dish plates for isolation of fungi. [4] Travel to orchards for sampling and harvesting is required for sampling and harvesting.

RECAP ORIGINAL OBJECTIVES

The overall objective of this study was to generate practical information regarding the impact on commercial cultivars of removal of inoculum from infected 'Manchurian' crabapple pollinizers in commercial apple orchards as part of a postharvest decay IPM program. Specific objectives were to:

1. Understand the in-season incidence of fruit infection by *Sphaeropsis pyriputrescens* and *Phacidiopycnis washingtonensis* following winter pruning of adjacent crabapple trees

2. Evaluate the impact of crabapple pruning on the incidence of Sphaeropsis rot and speck rot in commercial cultivars following storage

SIGNIFICANT FINDINGS

- Crabapple twig and mummy samples collected from all orchards with a history of speck rot or Sphaeropsis rot harbored viable pycnidia of the fungi before and at the time of pruning, indicating that they are one of the primary sources of inoculum for fruit infections in apples.
- *P. washingtonensis* infections and pycnidial formations were more prevalent on mummified crabapple fruit than twigs with dieback and canker symptoms, whereas such differences were not observed in the orchard infected by *S. pyriputrescens*.
- Fruit infections monitored during the 2013 growing season were highly correlated to the development of speck rots in storage, whereas the correlation was not apparent in 2014 season.
- Regardless the treatments, no *Sphaeropsis* infection was detected by a conventional isolation method from stem and calyx-end tissues of apple fruit during the growing season while Sphaeropsis rot developed in stored fruit, indicating that in-season monitoring for fruit infection by the isolation method may not predict the incidence of Sphaeropsis rot in storage.
- Regardless of the degree of pruning (chainsaw or detailed), pruning crabapple trees significantly reduced the incidence of speck rot and Sphaeropsis rot in apples during cold storage compared to the unpruned control.
- Crabapple fruit infected by *P. washingtonensis* or *S. pyriputrescens* during the growing season produced pycnidia before harvest, which provide secondary inocula for fruit infections within the growing season.

RESULTS & DISCUSSION

Inoculum availability of the fungi on crabapple trees

In 2013, crabapple twigs with dieback or canker symptoms and fruit mummies that had not been harvested in the previous year were sampled at the time of pruning crabapple trees before bloom. In all three orchards, pycnidia were observed on the twigs and fruit mummies, indicating that these were one of the primary sources of inoculum for fruit infection in the orchard. Pycnidial spores from all samples with pycnidia were microscopically examined and identified as *P. washingtonensis* according to the descriptions of Xiao et al. (2005). The morphological characteristics on potato dextrose agar (PDA) also confirmed that the fungus grown out from the pycnidial spores were *P.*

washingtonensis. All sampled crabapple trees, except one tree from orchard A that was recently replaced with a young tree, harbored viable pycnidia of the fungus at the time of pruning (Table 1). Percent samples with viable pycnidia ranged from 53 to 57% of the sampled twigs and 77 to 83% of the fruit mummies in these orchards. The fungus was isolated from 73 to 87% of the diseased twigs and 90 to 100% of the mummified crabapple fruit. In general, mummified crabapple fruit appeared to have higher percentages of *P. washingtonensis* infections and pycnidial formations than diseased twigs of crabapple trees.

In 2014, the presence of *S. pyriputrescens* was identified by sampling crabapple twigs with dieback or canker symptoms and mummified crabapple fruit in two blocks of 'Red Delicious' orchard with a history of Sphaeropsis rot incidences. Viability of pycnidial spores from all samples was evaluated by culturing the fungus on PDA. The identification of the cultures isolated from diseased tissues was confirmed according to the descriptions of *S. pyriputrescens* (Xiao and Rogers, 2004). All crabapple samples harbored viable pycnidia of the fungus at the time of pruning before and after bloom (Data not shown). This result indicates that infected crabapple trees are the primary source of inoculum for *Sphaeropsis* infections in the orchard.

Table 1. Identification and inoculum availability of *Phacidiopycnis washingtonensis* on crabapple trees at the time of pruning before bloom in three 'Red Delicious' orchards in central Washington

Orchard	Type of sample[a]	% Trees with viable pycnidia[b]	% Samples with viable pycnidia	% Samples with *P. washingtonensis*[c]
A	Twig	90.0	53.4 ± 7.4[d]	86.7 ± 5.4
	Mummy	90.0	83.3 ± 10.3	90.0 ± 10.0
B	Twig	100.0	53.3 ± 5.5	80.0 ± 5.4
	Mummy	100.0	76.7 ± 7.1	100.0 ± 0.0
C	Twig	100.0	56.7 ± 5.1	73.4 ± 6.7
	Mummy	100.0	83.4 ± 5.6	100.0 ± 0.0

[a] Three samples each for twigs with dieback or canker symptoms and mummified fruit of crabapple per tree, ten trees per orchard were randomly collected.
[b] Viability of pycnidial spores was assessed by plating conidia on acidified potato dextrose agar and examined for the growth of *P. washingtonensis*.
[c] Isolation of *P. washingtonensis* was made from diseased crabapple twigs and mummified fruit.
[d] Values are mean and standard error based on the samples from 10 trees.

In-season monitoring of fungal infections on apples

In 2013, detailed pruning that removed all infected twigs, branches, and mummified fruit of crabapple trees significantly reduced the infections of *P. washingtonensis* on apple fruit compared with the unpruned control and chainsaw pruning in all three orchards (Table 2). Although chainsaw pruning of crabapple trees was not as effective as detailed pruning, it significantly reduced fruit infections of apples compared to the unpruned control in two of three orchards. It is apparent that removing infected crabapple tissues by *P. washingtonensis* in the apple orchard can reduce the fruit infections on apple fruit during the fruit growing season. However, pruning alone could not completely prevent fruit infection on apples.

Regardless of pruning treatments, fruit infections in orchard A were generally higher than those in orchard B or C (Table 2). This might be due to the different types of irrigation; orchard A was irrigated by overhead sprinklers, and orchards B and C were irrigated by under-tree sprinklers. However, our study was not designed to test the effect of different irrigation methods.

In 2014, the trend of fruit infection by *P. washingtonensis* in orchard A and C was similar to that of 2013 although percent infections were numerically lower than the 2013 growing season (Tables 2 & 3). All crabapple trees in these orchards that were not selected for the study were chainsaw-pruned by the growers after bloom in 2014. No or very few infections of *P. washingtonensis* were detected on apple fruit adjacent to crabapple trees that were detail-pruned in 2013 without additional pruning in 2014, while a limited infection (1.3%) was detected in the chainsaw-detailed treatment, of which crabapple trees were chainsaw-pruned in 2013 and detail-pruned in 2014 (Table 3). In 2014, an orchard with a history of Sphaeropsis rot was added and detail-pruned before and after bloom. However, the entire block including unpruned controls was accidentally pruned by the grower after bloom. Therefore, an additional block with similar crabapple planting in the orchard was added to compare unpruned vs. commercially pruned (chainsaw) crabapple trees. Even though viable pycnidia of *S. pyriputrescens* were detected in all crabapple trees randomly sampled in this orchard, fruit infections during the growing season could not be detected by an isolation method from stem and calyx tissues of apples (Table 3).

At harvest time, we observed many crabapple fruit that were already infected by either *P. washingtonensis* or *S. pyriputrescens* and formed pycnidia on the fruit surface, which indicate that secondary inocula of the fungi are commonly present in the orchard for fruit infections near harvest (Fig. 2A). In 2014, newly developed cankers from infected crabapple fruit were observed, even on the trees that had all infected twigs and branches removed in the previous year (Fig. 2B). Therefore, crabapple fruit should be removed or harvested to reduce major sources of inoculum on crabapple trees.

Fig. 1. Crabapple fruit infected and formed pycnidia (fruiting bodies of the fungus) by *Phacidiopycnis washingtonensis* at the time of apple harvest in 2014 (A) and a canker originated from infected crabapple fruit on the tree that was detail-pruned in 2013 (B).

Development of decays in storage

In a 2-year study, pruning crabapple trees that involved removing infected twigs and branches significantly and consistently reduced the incidence of speck rot in apples during cold storage compared to the unpruned control (Tables 2 & 3). Only a one-year trial was conducted in orchard B,

and no statistical difference among the treatments was observed, probably due to the non-uniformity of crabapple planting in this orchard (Table 2). Detailed pruning significantly reduced the fruit infections during growing season compared to the chainsaw pruning, but the difference was not significant in the development of speck rots in storage. It is apparent that removing infected tissues of crabapple trees by pruning reduces the incidence of speck rot in stored apples. As we observed in fruit infection monitoring during the season, we could not demonstrate a complete control of speck rot on apples by pruning crabapple trees. Similar to the fruit infection in the orchard, the incidence of speck rot was also higher in orchard A than orchard B or C, probably due to the use of overhead sprinklers in orchard A (Tables 2 & 3). Therefore, overhead irrigation should be avoided.

Table 2. Incidence of fruit infection in the orchard and speck rot in storage caused by *Phacidiopycnis washingtonensis* on apples adjacent to crabapple trees in three commercial 'Red Delicious' orchards in 2013-2014 season

		Incidence of *P. washingtonensis* (%)[y]	
Orchard	Treatment	Infection in-season	Speck rot in storage[z]
A	Unpruned	25.0 a	23.5 a
	Chainsaw	10.0 b	16.6 ab
	Detailed	5.6 c	7.8 b
B	Unpruned	10.6 a	8.1 a
	Chainsaw	4.4 ab	5.9 a
	Detailed	3.8 c	3.1 a
C	Unpruned	10.6 a	11.6 a
	Chainsaw	5.6 b	3.4 b
	Detailed	1.9 c	2.5 b

[y] Eighty apples per replicate, 4 replications per treatment were harvested and stored for up to 9 months at 4°C.

[z] Values within an orchard and column, when followed by a common letter, are not significantly different according to the analysis of variance and least significant difference ($P = 0.05$).

To evaluate the effect of crabapple pruning on the incidence of Sphaeropsis rot in storage, apples were harvested from two blocks of 'Red Delicious' orchard (Orchard D1 and D2) after pruning crabapple trees before and after bloom. In orchard D1, all crabapple trees including the unpruned control were accidentally pruned; therefore no data was obtained for the unpruned control. All three pruned treatments showed low incidences of Sphaeropsis rot, although the incidence of detailed pruning after bloom was lower than those of either detailed pruning before bloom or chainsaw pruning after bloom (Table 3). In an additional block (D2) selected to replace the block D1, chainsaw pruning after bloom significantly reduced the incidence of Sphaeropsis rot in storage. This study clearly demonstrated that regardless of the pruning levels, crabapple pruning in commercial apple orchards resulted in consistently lower development of speck rot and Sphaeropsis rot in storage.

Correlation between fruit infection and speck rot development in storage

To analyze the relationship between the fruit infection monitored during the fruit growing season by isolating *P. washingtonensis* on stems and sepals of apple fruit and speck rot development during cold storage, Pearson correlation analysis was performed. There was significant correlation between the fruit infection by *P. washingtonensis* during the growing season and the decay development in storage

in all three orchards (P <0.05) in 2013-2014 season (Fig. 2), whereas a weak (orchard C) or no correlation (orchard A) was observed in 2014-2015 season.

Table 3. Incidence of fruit infection in the orchard and decays in storage caused by *Phacidiopycnis washingtonensis* and *Sphaeropsis pyriputrescens* on apple fruit adjacent to crabapple trees in commercial 'Red Delicious' orchards in 2014-2015 season

Orchard	Pathogen	Treatment[x]	Infection in-season (%)	Decay in storage (%)[z]
A	*P. washingtonensis*	Unpruned	6.3 a[y]	31.3 a
		Chainsaw-detailed	1.3 a	3.1 b
		Detailed-unpruned	0.0 a	0.6 b
C	*P. washingtonensis*	Unpruned	4.4 a	15.3 a
		Chainsaw-detailed	1.3 ab	3.0 b
		Detailed-unpruned	0.6 c	0.9 b
D1[w]	*S. pyriputrescens*	Unpruned-Chainsaw	0.0	2.0 a
		Detailed before bloom	0.0	1.7 a
		Detailed after bloom	0.0	0.2 b
D2	*S. pyriputrescens*	Unpruned	0.0	15.5 a
		Chainsaw	0.0	3.9 b

[w] All crabapple trees in orchard D1 were accidentally pruned by the grower after pruning treatments.
[x] Crabapple trees that were not selected for the study in all orchards, but D2 were chainsaw-pruned by the growers after bloom. Trees in chainsaw-detailed treatment were chainsaw-pruned in 2013 followed by detailed pruning in 2014. Trees in detailed-unpruned treatment were detailed pruned in 2013 and unpruned in 2014.
[y] Values within an orchard, when followed by a common letter, are not significantly different according to the analysis of variance and least significant difference (P = 0.05).
[z] One hundred sixty apples per replicate, 4 replications per treatment were harvested and stored for up to 9 months at 4°C.

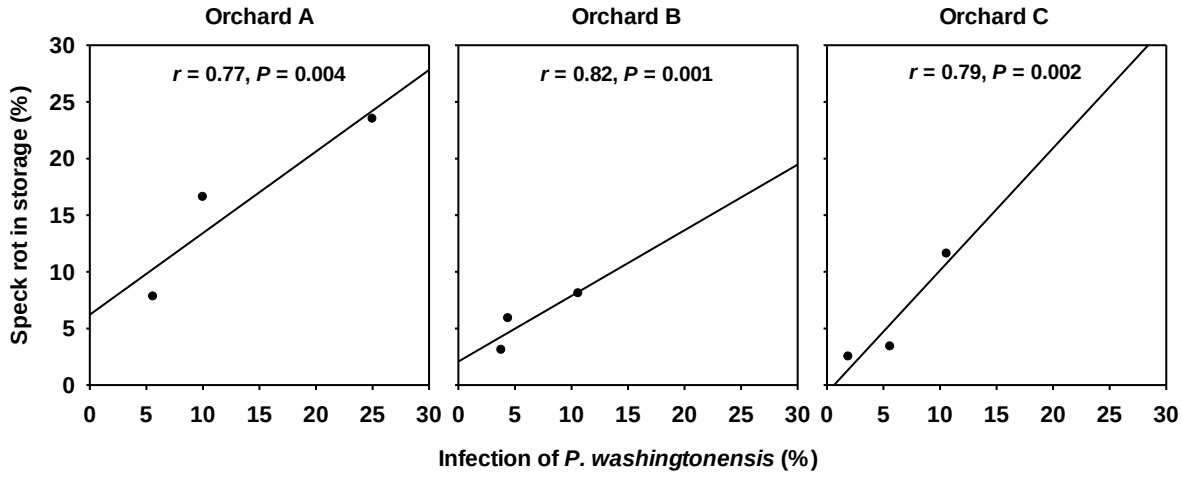

Fig. 2. Correlation between the infection of *Phacidiopycnis washingtonensis* on stems and sepals of apple fruit adjacent to crabapple trees during fruit growing season and speck rot development during cold storage harvested from three commercial 'Red Delicious' orchards in 2013-14 season.

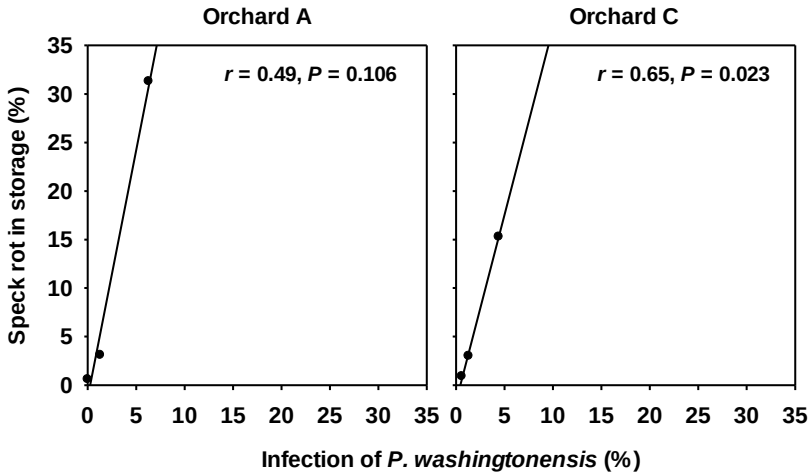

Fig. 3. Correlation between the infection of *Phacidiopycnis washingtonensis* on stems and sepals of apple fruit adjacent to crabapple trees during fruit growing season and speck rot development during cold storage harvested from two commercial 'Red Delicious' orchards in 2014-15 season.

REFERENCES CITED

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EXECUTIVE SUMMARY

This study was designed to generate practical information regarding the impact of 'Manchurian' crabapple pruning in commercial apple orchards to reduce or control speck rot and Sphaeropsis rot of apple in storage. To demonstrate the importance of crabapple pruning, two specific objectives were addressed: 1) monitoring the incidence of fruit infections by *Phacidiopycnis washingtonensis* and *Sphaeropsis pyriputrescens* on apples adjacent to crabapple trees after various pruning treatments before or after bloom and 2) evaluating the development of speck rot and Sphaeropsis rot on apples in cold storage for up to 9 months.

In all orchards selected for this study, crabapple twigs and fruit mummies harbored viable pycnidia of the fungi before and at the time of pruning, indicating that they are the primary sources of inoculum for fruit infections in apples. In particular, fruit mummies left hanging on the trees formed more fruiting bodies (pycnidia) of the fungi containing millions of spores than twig diebacks and cankers. Moreover, infected crabapple fruit during growing season also produced pycnidia on the tree that serve as inoculum throughout the remainder of the season and caused twig cankers in the following season, further supporting the impotence that infected crabapples should be removed or harvested before the formation of pycnidia on the fruit.

The correlation between fruit infection monitored during fruit growing season and the development of speck rot in storage was relatively strong in one year, but not apparent in the following season. Therefore, monitoring fruit infections by isolating the fungus from stem and calyx tissues could not predict the incidence of speck rot on apples in storage. Similarly no *Sphaeropsis* infection was detected by a conventional isolation method from stem and calyx-end tissues of apple fruit during the growing season while Sphaeropsis rot developed in stored fruit.

In this study, pruning crabapple trees significantly reduced the incidence of speck rot and Sphaeropsis rot in apples during cold storage regardless of the degree of pruning (chainsaw or detailed). Although we could not demonstrate the difference between pruning before bloom and after bloom, it is clear that removal of diebacks and cankers of twigs and branches in the beginning of the season would help reduce the inoculum levels of the fungi in the orchard. Therefore, we clearly demonstrated that crabapple pruning should be implemented as part of a postharvest decay IPM program. As shown in this study, since pruning treatment alone could not completely prevent the development of speck rot and Sphaeropsis rot, additional treatments such as pre-harvest sprays with appropriate fungicides or/and postharvest fungicides are highly recommended.