FINAL REPORT

WTFRC Project #AH-04-422 **WSU Project # 13C-3661-4368**

Project title: Sphaeropsis rot in apple

PI: Chang-Lin Xiao, Assistant Plant Pathologist
Organization: WSU Tree Fruit Research and Extension Center
Address, phone, e-mail: 1100 N. Western Avenue, Wenatchee; 509-663-8181 ext. 229; clxiao@wsu.edu

Cooperators: Yong-Ki Kim, Postdoctoral Researcher, WSU, Wenatchee
Dana Faubion, WSU, Yakima
Packinghouses in major apple production areas

Objectives:
1. Determine the prevalence and incidence of Sphaeropsis rot as well as other postharvest diseases on major apple varieties under various handling systems (traditional drench, MCP, etc.).
2. Determine the sources of inoculum of the Sphaeropsis fungus in the orchard.
3. Test sensitivity of the fungus to various fungicides.
4. Evaluate effectiveness of preharvest fungicides and postharvest drench in controlling Sphaeropsis rot.

Significant findings:
- In addition to Red Delicious, Sphaeropsis rot also has the potential to cause significant economic losses on Fuji. In one instance, 11% of the fruit in storage bins were rotted by Sphaeropsis rot in March. Sphaeropsis rot has been observed on Red Delicious, Golden Delicious, Fuji, Granny Smith and Gala grown in different apple-producing areas in the state.
- Of the 78 grower lots sampled in a large-scale survey in 2004, 60 had Sphaeropsis rot. Sphaeropsis rot was an important component of the storage decay complex and could cause significant losses during storage. Overall, Sphaeropsis rot accounted for an average of 18-19% of the decay on Fuji and Golden Delicious and 22% of the decay on Red Delicious.
- Sphaeropsis rot was an orchard-related postharvest disease. Infection of fruit by the Sphaeropsis fungus occurred in the orchard and symptoms developed during storage. Further research is needed to determine timing of infection.
- The fungus *Sphaeropsis pyriputrescens* was associated with a twig dieback and canker disease of apple and crabapple trees in apple orchards. In 2004, we observed that the Sphaeropsis fungus was associated with twig dieback, cankers or dead tissues on trees of Fuji and Red Delicious. In a Fuji orchard with a history of severe Sphaeropsis rot during storage, 100% of the crabapple trees and 43% of the apple trees were infected by the Sphaeropsis fungus. This indicates that both infected apple and crabapple trees in the orchard may serve as important sources of inoculum of the Sphaeropsis fungus.
- On infected Fuji trees, *Sphaeropsis pyriputrescens* was commonly associated with dead fruit spurs, dead pedicle of flowers or fruit, and twigs with dieback symptoms or cankers. On infected Red Delicious trees, the fungus was found on dead fruit spurs or bark but at a low frequency. These infected tissues could be important sources of inoculum, but their relative importance as a primary source of inoculum may be orchard-dependent. Further research is needed to understand the life cycle of the fungus in the orchard.

- It appeared that viable inoculum of the Sphaeropsis fungus was available throughout the apple-growing season, at least in one Fuji orchard. Further research is needed to monitor availability of inoculum for fruit infection.
- The fungus *Sphaeropsis pyriputrescens* exhibited varying degrees of sensitivity to various fungicides. Broad-spectrum fungicides Captan, Dithane and Ziram were effective in inhibiting both mycelial growth and spore germination only at high rates. *Sphaeropsis pyriputrescens* exhibited different patterns of sensitivity to Procure (a DMI fungicide) and Flint (a strobilurin). Differential responses of *Sphaeropsis pyriputrescens* to common pre- and postharvest fungicides raised interesting questions about strategies of using different classes of fungicides in the orchard or as postharvest treatments for control of Sphaeropsis rot.

Methods:

In 2004, we collected decayed apple fruit from commercial packinghouses in major fruit production areas in Washington State. To determine the incidence of rots caused by the Sphaeropsis fungus and other decay-causing pathogens, approximately 50 decayed fruit from each grower lot were randomly sampled. A total of 78 grower lots were sampled. Decay was categorized by causal agents through examining symptoms, presence of sporulation of the pathogen and/or isolating from the diseased tissue.

Sources and availability of inoculum of the Sphaeropsis fungus were monitored in two apple orchards (one Red Delicious and one Fuji). During the apple growing season we sampled the following materials from each orchard at approximately 6-week intervals: 1) twigs with symptoms of dieback or canker from crabapple trees; 2) twigs with dieback symptoms or cankers, if present, from apple trees; and 3) dying or dead bark or twigs on apple trees. These samples were examined under a microscope for the presence of fruiting bodies of the fungus. When necessary, isolations of causal agents from diseased tissues were also performed.

The currently labeled fungicides for apple, including Captan, Dithane, Flint, Mertect, Procure and Ziram, were amended in an agar medium at different concentrations to test fungicide sensitivity of the fungus. Isolates of the fungus recovered from different geographical regions were included in the fungicide sensitivity tests. Effectiveness of fungicides in inhibiting mycelial growth and spore germination were evaluated.

An experiment was conducted in a commercial orchard of Red Delicious with a history of severe Sphaeropsis rot to evaluate effectiveness of preharvest fungicides and postharvest drench with Mertect in controlling Sphaeropsis rot. Flint and Ziram were applied at two weeks before harvest and Thiram at one week before harvest. Part of the fruit from the nontreated control were drenched with Mertect before storage. Fruit were stored in CA.

Results and discussion

<u>Prevalence and incidence of Sphaeropsis rot and other diseases.</u> In 2004, we sampled decayed fruit from 78 different grower lots (39 lots of Red Delicious, 19 lots of Golden Delicious, 14 lots of Fuji and 3 lots each of Gala and Granny Smith) from packinghouses across the sate. Gray mold, blue mold and Sphaeropsis rot were the three major postharvest diseases on Red Delicious and Fuji, accounting for 24, 41 and 22% of the total decay on Red Delicious, respectively, and 32, 30 and 19% of the total decay on Fuji, respectively (Fig. 1). On Golden Delicious, in addition to these three diseases, Bull's eye rot was also an important component of decay; gray mold, blue mold, Sphaeropsis rot and Bull's eye rot accounted for 30, 19, 18 and 30% of the total decay, respectively. Among the 78 grower lots including all five varieties, gray mold, blue mold, Sphaeropsis rot and Bull's eye rot accounted for 29, 34, 19 and 10% of the total decay, respectively (Fig. 1).

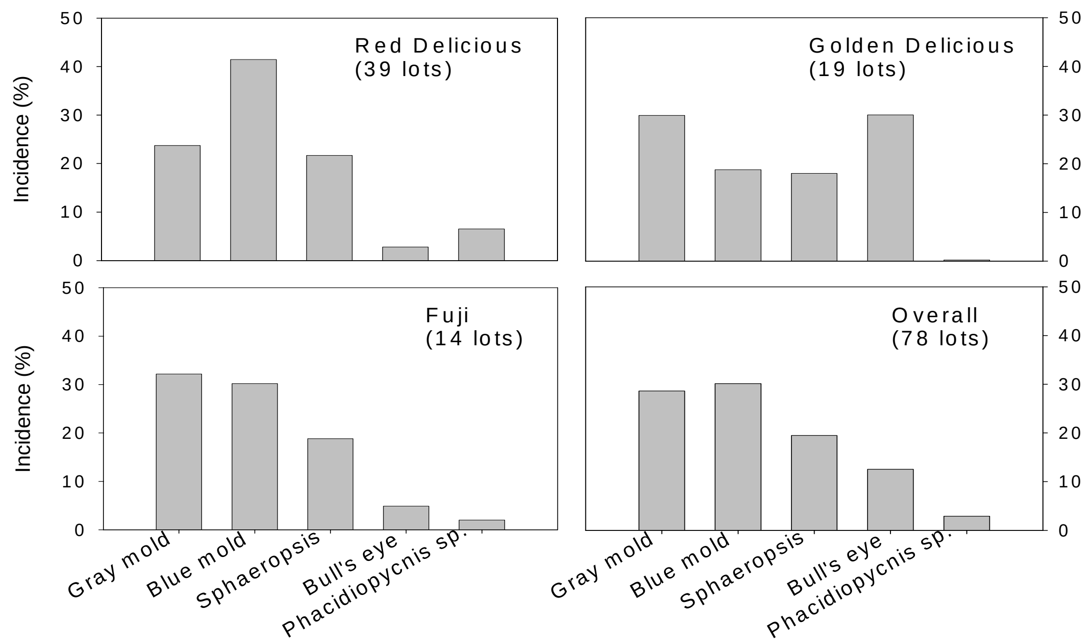

Fig. 1. Percentage of decay caused by various pathogens on different apple varieties.

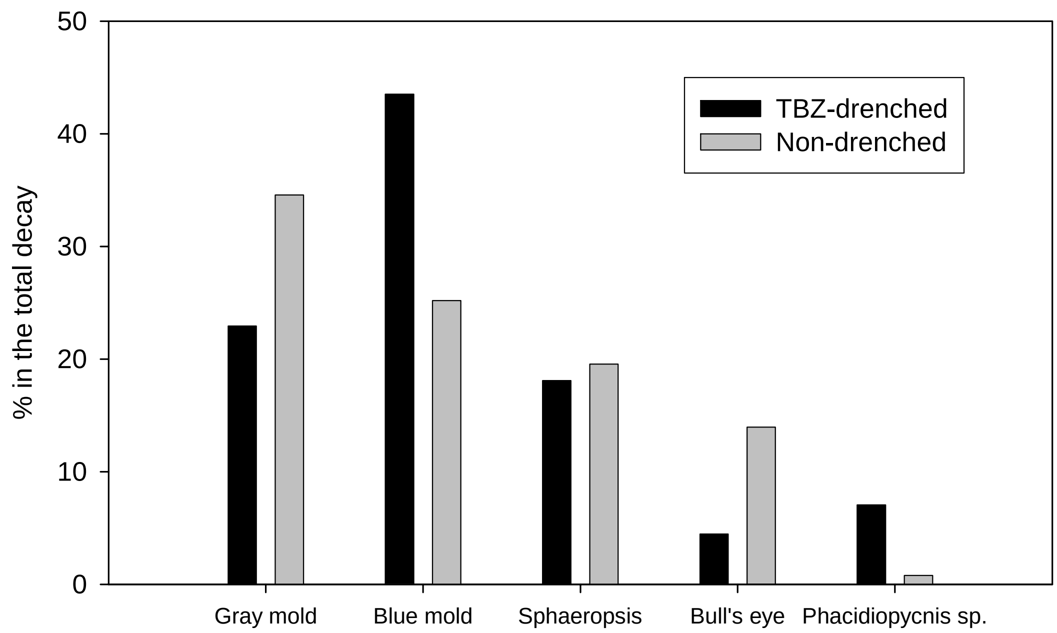

Fig. 2. Percentage of decay caused by various pathogens in TBZ-drenched and non-drenched grower lots.

Sphaeropsis rot occurred in all Red Delicious and Golden Delicious lots sampled in 2004 and in nine of the 14 Fuji lots. Percentage of the total decay caused by the Sphaeropsis fungus varied from orchard to orchard. We have observed high percentages of Sphaeropsis rot on Red Delicious, Golden Delicious and Fuji. In 2004, in addition to instances of severe Sphaeropsis rot on Red Delicious, we have also observed an instance of severe Sphaeropsis rot on Fuji in which 11% of the fruit were rotted by *Sphaeropsis pyriputrescens* in March. Consequently, we chose the Fuji orchard with a history of severe Sphaeropsis rot for further study to understand the orchard phase of this disease.

Of the 78 grower lots sampled in 2004, 45 were drenched with Mertect. Sphaeropsis rot accounted for 18 and 20% of the total decay on TBZ-drenched and non-drenched fruit, respectively (Fig. 2). Gray mold was dominant on non-drenched fruit, whereas blue mold was dominant on TBZ-drenched fruit. A postharvest fruit rot caused by *Phacidiopycnis* sp. was observed on some lots. The higher incidence of the decay caused by *Phacidiopycnis* sp. on TBZ-drenched fruit than non-drenched fruit was because two samples from TBZ-drenched fruit had an extremely high incidence of *Phacidiopycnis* sp.

<u>Sources and availability of inoculum in the orchard.</u> The 2004 study was conducted in two orchards. It appears that sources of inoculum in these two orchards were somewhat different. In the Fuji orchard, we found that the following sources likely played an important role in initiating disease:

- *Cankers and twig dieback of crabapple trees*. During the 2004 growing season, 50 twigs with dieback symptoms were sampled from crabapple trees in the Fuji orchard. All sampled crabapple trees were infected by the Sphaeropsis fungus. The vast majority of crabapple trees in this orchard were Manchurian crabapples.
- *Twig dieback and dead fruit pedicle on Fuji apple trees*. On August 10, 40 Fuji trees were surveyed for presence of the Sphaeropsis fungus; 58% of the trees had pycnidia of the fungus on dead fruit spurs or twigs with dieback symptoms. The fungus was recovered from 43% of the trees based on isolation of the fungus from diseased tissues.
- *Fuji fruit mummies on the trees*. In the Fuji orchard, of the 30 Fuji mummies sampled from the trees on May 18, 2004, 13 had pycnidia of the Sphaeropsis fungus, accounting for 43% of the mummies. Pycnidia that formed on mummies contained viable conidia of the fungus.

The results indicate that dieback twigs of crabapple trees, dead fruit spurs and mummies on Fuji trees can provide inoculum for infection of fruit or trees in the orchard.

Table 1 is the summary of inoculum availability during the fruit-growing season in two apple orchards. The results indicate that viable inoculum of the Sphaeropsis fungus was available throughout the growing-season in the Fuji orchard.

In the Red Delicious orchard, crabapple trees were not commonly planted. Although pycnidia of the Sphaeropsis fungus were observed on crabapple trees in this orchard, crabapple trees were likely not the major source of inoculum. Thus, in this orchard the monitoring was focused on dying or dead tissues on Red Delicious apple trees. We sampled dead fruit spurs or twigs and dead bark. We did observe *Sphaeropsis pyriputrescens* on these samples but at a relatively low frequency (Table 1). Given the fact that we have observed high incidences of fruit infected by the fungus from this orchard in two consecutive years (2003 and 2004), it is expected that the fungus is commonly present in this orchard. It is unclear whether some other sources may play a role in infection of fruit in this particular orchard. One possibility is that a low percentage of dead tissues on the trees is infected by the fungus, but the amount of inoculum present on the trees is sufficient to cause considerable infection of fruit. The other possibility is that the teleomorph state (sexual state of the fungus) might be involved in the disease cycle. Sexual spores of the fungus likely can be dispersed by air or wind based on the biology of fungi closely related to the Sphaeropsis fungus.

Table 1. Sources and availability of inoculum of the Sphaeropsis fungus in two apple orchards.

Date	Orchard	Variety	Type of samples[1]	%Trees with pycnidia	%Samples with pycnidia
10-May	1	Crabapple	Twigs	100	100
		Fuji	Spurs/Twigs	50	35.7
	2	Red Delicious	Bark	30	4
			Spurs		
21-Jun	1	Crabapple	Twigs	100	100
		Fuji	Spurs/Twigs	70	30
	2	Red Delicious	Bark	0	0
			Spurs	10	3.3
10-Aug	1	Crabapple	Twigs	100	80
		Fuji	Spurs/Twigs	58	30.8
	2	Red Delicious	Bark	20	2
			Spurs	40	13.3
24-Sep	1	Crabapple	Twigs	100	93.3
		Fuji	Spurs/Twigs	50	20
	2	Red Delicious	Bark	0	0
			Spurs	0	0

[1] In the Fuji orchard, at each sampling time, 3 dieback twigs from each of 10 crabapple trees and 3 dead fruit spurs or twigs from each of 10 Fuji trees were sampled. In the Red Delicious orchard, 3 dead fruit spurs and 10 pieces of dead bark tissues from each of 10 trees were sampled.

It was a good approach to monitor sources and availability of inoculum in two different orchards. Our results suggested that crabapple and infected fruit spurs and dead bark on apple trees can be important sources of inoculum, but their relative importance as a primary source of inoculum may be orchard-dependent. More research is needed to gain a better understanding of the disease cycle in order to develop relevant measures for control of Sphaeropsis rot.

<u>Sensitivity of *Sphaeropsis pyriputrescens* to various fungicides.</u> Sensitivity of mycelial growth and conidial germination to selected fungicides were tested in vitro, and 10 isolates recovered from different sources (6 from decayed apple fruit, 1 from a Fuji tree, 1 from a crabapple tree and 2 from decayed d'Anjou pear fruit) in different production areas in central Washington were included in the tests. Ziram was effective in inhibiting both mycelial growth and conidial germination. The other two broad-spectrum fungicides, Captan and Dithane, were effective at high rates (label rates and $1/10^{th}$ of the label rates) (Tables 2 and 3). Two classes of fungicides commonly used in apple orchards, DMIs (Procure) and Strobilurins (Flint), were tested. *Sphaeropsis pyriputrescens* exhibited different patterns of sensitivity to these two fungicides. Procure was very effective in inhibiting mycelial growth, even at 10^{-3} label rate but not effective in inhibiting conidial germination. Flint was not effective in inhibiting mycelial growth but was very effective in inhibiting conidial germination. The postharvest fungicide Mertect (thiabendazole) was effective in inhibiting mycelial growth. Mertect is a systemic fungicide. We observed that Mertect did not inhibit conidial germination but inhibited elongation of germ tubes. Differential responses of the Sphaeropsis fungus to DMI and Strobilurin fungicides and Mertect raised interesting questions about strategies of using different classes of fungicides in the field or as postharvest treatments for control of Sphaeropsis rot. We observed variation in sensitivity among the isolates, particularly for spore germination. Further research is needed to advance our understanding.

Our preliminary study indicated that a postharvest drench with thiabendazole provided a good control of Sphaeropsis rot. In 2004, two new postharvest fungicides, fludioxonil (Scholar) and pyrimethanil (Penbotec) belonging to two new classes of fungicides, were registered for use on pome fruits for control of postharvest diseases; research needs to be done to evaluate whether a postharvest treatment with either Penbotec or Scholar is effective in controlling Sphaeropsis rot.

Table 2. Effectiveness of different classes of fungicides in inhibiting in vitro mycelial growth of *Sphaeropsis pyriputrescens*.

			Inhibition (%)	
Fungicide	Class	Concentration	Range[1]	Average
Captan	Multi-site activity	X (label rate)	99.5−100	99.9
		10^{-1} X	75.8−100	94
		10^{-2} X	0	0
		10^{-3} X	0	0
		10^{-4} X	0	0
Dithane	Multi-site activity	X (label rate)	100	100
		10^{-1} X	99.5−100	99.9
		10^{-2} X	0−43.9	19.3
		10^{-3} X	0	0
		10^{-4} X	0	0
Flint	Strobilurin	X (label rate)	16.5−67.5	40.6
		10^{-1} X	19.8−59.2	37.9
		10^{-2} X	13.6−61.3	35.7
		10^{-3} X	10.7−44.4	23.8
		10^{-4} X	6.7−32.5	17.2
Procure	DMI	X (label rate)	100	100
		10^{-1} X	100	100
		10^{-2} X	95.3−99.5	97.9
		10^{-3} X	87.3−98.5	93.5
		10^{-4} X	51.2−75.2	59.7
Mertect	Benzimidazole	X (label rate)	100	100
		10^{-1} X	95.8−100	99.6
		10^{-2} X	99.5−100	99.9
		10^{-3} X	89.3−100	94.5
		10^{-4} X	92.2−100	98.3
Ziram	Multi-site activity	X (label rate)	100	100
		10^{-1} X	98.3−100	99.8
		10^{-2} X	64.6−82.6	76.1
		10^{-3} X	69.4−88.3	79.2
		10^{-4} X	9.7−75.6	49.5

[1] Ten isolates were included in the test. Ranges and averages of sensitivity to each fungicide are presented.

Table 3. Effectiveness of different classes of fungicides in inhibiting conidial germination of *Sphaeropsis pyriputrescens*.

Fungicide	Class	Concentration	Inhibition (%)	
			Range[1]	Average
Captan	Multi-site activity	X (label rate)	100	100
		10^{-1} X	100	100
		10^{-2} X	0−100	46.4
		10^{-3} X	0−45.7	13.7
		10^{-4} X	0−18.2	5.5
Dithane	Multi-site activity	X (label rate)	100	100
		10^{-1} X	25.5−100	90.3
		10^{-2} X	7.3−63.2	36.9
		10^{-3} X	0−38.2	12.1
		10^{-4} X	0−21.1	3.7
Flint	Strobilurin	X (label rate)	100	100
		10^{-1} X	100	100
		10^{-2} X	99.3−100	99.7
		10^{-3} X	76−99	89
		10^{-4} X	2.8−58.4	22.5
Procure	DMI	X (label rate)	51−100	82.6
		10^{-1} X	0−28.1	11.2
		10^{-2} X	0−5.6	1.3
		10^{-3} X	0−17.5	4
		10^{-4} X	0−24.7	5.9
Mertect	Benzimidazole	X (label rate)	1.2−63.6	34.7
		10^{-1} X	0−41.5	18.4
		10^{-2} X	0−24.6	6.2
		10^{-3} X	0−25.3	10.4
		10^{-4} X	0−41.1	8.4
Ziram	Multi-site activity	X (label rate)	100	100
		10^{-1} X	100	100
		10^{-2} X	18.1−94.1	58.1
		10^{-3} X	22.8−99.5	56
		10^{-4} X	12.1−87.3	43.9

[1] Ten isolates were included in the test. Ranges and averages of sensitivity to each fungicide are presented.

Control of Sphaeropsis rot. In 2004, an experiment was conducted in a Red Delicious orchard with a history of Sphaeropsis rot to evaluate preharvest and postharvest fungicides for control of Sphaeropsis rot. The fruit are still in a CA storage. Decay will be evaluated in May 2005, and results will be forthcoming.

Budget:

Project title: Sphaeropsis rot in apple
PI: C. L. Xiao
Project duration: One year
Current year: 2004
Original budget request: $37,288

Year	**2004**
Total	**$37,288**

Current year breakdown:

Item	**2004**
Salaries[1]	$22,838
Benefits (37%)	8,450
Wages	
Benefits	
Equipment	
Supplies [2]	3,000
Travel [3]	3,000
Miscellaneous	
Total	$37,288

[1] Nine-month salary for Dr. Y. K. Kim, Postdoctoral Research Associate.

[2] Isolation media, chemicals, petri dish plates, cryogenic vials and other supplies for ultra-low temperature storage of fungal isolates, and fungicides. Cost of fruit bought from commercial orchards. Cell phone charges are allowed on this grant.

[3] Travel twice weekly to packinghouses across the state was required for sampling from March to August. We used a leased vehicle.