Sources and Availability of Inoculum and Seasonal Survival of *Sphaeropsis pyriputrescens* in Apple Orchards

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Abstract

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Sphaeropsis pyriputrescens is the cause of Sphaeropsis rot, a recently reported postharvest fruit rot disease of apple. Infection of apple fruit by the fungus is believed to occur in the orchard, and symptoms develop during storage or in the market. *S. pyriputrescens* also is the cause of a twig dieback and canker disease of apple and crabapple trees. To determine sources of pathogen inoculum in the orchard, twigs with dieback and canker symptoms, dead fruit spurs, dead bark, and fruit mummies on the trees were collected and examined for the presence of pycnidia of *S. pyriputrescens*. To monitor inoculum availability during the growing season from early May to early November, dead fruit spurs or twigs from Fuji trees, and twigs with dieback from crabapple trees (as a source of pollen for apple production) in a Fuji orchard as well as dead fruit spurs and dead bark from Red Delicious trees in a Red Delicious orchard were sampled periodically and examined for the presence and viability of pycnidia of *S. pyriputrescens*. To determine seasonal survival and production of pycnidia of the fungus on twigs, apple twigs were inoculated in early December, sampled

periodically for up to 12 months after inoculation, examined for the presence of pycnidia, and subjected to isolation of the fungus from diseased tissues to determine its survival. Pycnidia of *S. pyriputrescens* were observed on diseased twigs, dead fruit spurs and bark, and mummified fruit on both apple and crabapple trees, suggesting that these tissues were the sources of inoculum for fruit infection in the orchard. With the combined observations from two orchards during three growing seasons, viable pycnidia of the fungus were present throughout the year and observed in 50 to 100% of the Fuji trees, >90% of crabapple trees, and 0 to 50% of the Red Delicious trees. *S. pyriputrescens* was recovered from diseased tissues of inoculated twigs at all sampling times up to 12 months after inoculation. The results suggest that *S. pyriputrescens* can survive as mycelium in diseased twigs in north-central Washington State and that availability of viable *S. pyriputrescens* pycnidia is unlikely a limiting factor for infection of apple fruit in the orchard leading to Sphaeropsis rot during storage.

Washington State leads apple production in the United States, with a 2011 value of \$1.83 billion (10). After harvest, apples are either packed and shipped to the market or stored at 0 to 4°C in air or in controlled atmosphere (1 to 2% O₂ and 0.5% CO₂) at –1 to 3°C (9) and packed for up to 12 months. While modern postharvest refrigeration and technology allow the industry to store fruit for an extended period of time and still provide quality fruit to consumers, postharvest fruit rot diseases, if left uncontrolled, can cause significant economic losses during storage or in the market (20).

Sphaeropsis rot, caused by *Sphaeropsis pyriputrescens* Xiao & J.D. Rogers, is a recently reported postharvest fruit rot disease of apple and pear in the United States (21,22). A survey conducted in Washington State showed that Sphaeropsis rot accounted for an average of 17% in the total decayed fruit of 'Red Delicious', 'Golden Delicious', and 'Fuji' apples (5). In one instance, 24% of the Red Delicious apples harvested from a commercial orchard that did not receive pre- or postharvest fungicide treatments were decayed by *S. pyriputrescens* 9 months after harvest (22). Inoculation of Red Delicious, Golden Delicious, and Fuji apple fruit in the orchard showed that *S. pyriputrescens* infects the stem, floral parts,

and lenticels of fruit; infections can occur at different times from 3 weeks after petal fall to near harvest but remain latent; and symptoms of Sphaeropsis rot develop only after harvest, indicating that Sphaeropsis rot is an orchard-originated postharvest disease (4,5).

S. pyriputrescens also is the cause of a canker and twig dieback disease of apple and crabapple trees (18). In Washington State, crabapple is commonly used as a source of pollen in commercial apple production and may account for 5 to 10% of the trees in apple orchards. Pycnidia of *S. pyriputrescens* are often present on diseased crabapple twigs in the orchard (18). The pycnidia that develop on diseased twigs of crabapple and apple may serve as inoculum for infection of apple fruit leading to Sphaeropsis rot in storage. As Sphaeropsis rot originates from infections of apple fruit in the orchard, sources and availability of viable inoculum in the orchard likely play an important role in initiating infections.

The objectives of this study were to determine (i) the sources and availability of viable inoculum of *S. pyriputrescens* during the fruit-growing season in apple orchards and (ii) seasonal survival and pycnidial production of *S. pyriputrescens* on diseased twigs of apple trees under orchard conditions.

Materials and Methods

Sources of inoculum. This study was conducted in one commercial Fuji apple orchard, designated Morris orchard, and one Red Delicious apple orchard, designated Markel orchard, both located in Manson, WA. These two orchards were selected for this study because significant losses of fruit from these orchards resulting from Sphaeropsis rot were observed in 2003 (22; C. L. Xiao, *personal observations*). In Morris orchard, crabapple trees ('Manchurian') were planted as pollinizers intermixed with Fuji apple trees (10 apple trees between two crabapple trees within a row). In Markel orchard with Red Delicious, Golden Delicious trees were planted as pollinizers and only very few crabapple trees were planted (less than 10 crabapple trees in a 2.8-ha orchard block).

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To determine sources of *S. pyripitrescens* inoculum in the orchard, twigs with dieback and canker symptoms, dead fruit spurs, or bark on the trees were collected in September 2003 and in April to May 2004 in both orchards. Samples were examined under a dissecting microscope for the presence of *S. pyripitrescens* pycnidia following the published descriptions of the fungus (18,21). If pycnidia similar to those of *S. pyripitrescens* were present on the samples, selected pycnidia were then crushed in sterile water and examined under a microscope, and drops of conidia were streaked on acidified potato dextrose agar (APDA; Difco Laboratories, Detroit, MI) (4 ml of a 25% solution of lactic acid per liter of medium) to establish cultures of the fungus. To attempt to isolate the fungus from diseased twigs, outer bark tissues of diseased twigs were scraped and small segments were cut from the margins between the diseased and healthy tissues. Tissue segments were surface-sterilized for 5 min in 0.5% sodium hypochlorite solution, rinsed three times with sterile water, cut into small pieces, and placed on APDA. Isolation plates were incubated at 20°C in the dark for up to 10 to 14 days. Identification of *S. pyripitrescens* was based on the published descriptions of the fungus (15,18,21).

Mummified Fuji apple fruit hung on the apple trees were observed in Morris orchard in springs of both 2005 and 2006. Black pycnidia were evident on some mummies. To determine whether *S. pyripitrescens* pycnidia were present on Fuji mummies, three Fuji mummies from each of 10 trees that had mummies were collected

in Morris orchard on 13 May 2005 and 19 May 2006. Mummies were examined for the presence of *S. pyripitrescens* pycnidia under a dissecting microscope. Pycnidia were examined under a microscope and conidia were plated on APDA as described above.

Manchurian crabapple fruit mature earlier than Fuji apple fruit, but crabapple fruit are commonly left on the trees. We observed fruit rots on crabapple fruit during the Fuji apple harvest period in Morris orchard. On 26 October 2004, 14 crabapple trees were sampled for fruit rots, and 10 to 21 crabapple fruit with visible rot symptoms were collected from each tree. Diseased fruit were first examined under a dissecting microscope for the presence of *S. pyripitrescens* pycnidia as described above. After examination, diseased fruit were lightly sprayed with 70% ethanol and allowed to dry. The skin of the diseased area on the fruit was peeled off at the margin of decayed and healthy tissue using a sterile scalpel, and then small fragments of decayed flesh were cut and plated on APDA. Plates were incubated at room temperature (20 to 22°C) for up to 10 to 14 days for culture development.

Availability of inoculum. Availability of viable inoculum of *S. pyripitrescens* was monitored in both Morris and Markel orchards from 2004 to 2006. Sampling started early May through early November at approximately 4-to-6-week intervals (Tables 1 and 2). In Morris orchard, 10 crabapple trees and 10 Fuji apple trees were selected and marked with plastic ribbons to avoid resampling the same trees during the course of the study. Selected trees were at

Table 1. Inoculum availability of *Sphaerospora pyripitrescens* in a commercial 'Fuji' apple orchard in Manson, Washington State

Year	Sampling date	Variety ^a	% Trees with viable pycnidia ^v	% Samples from which <i>S. pyripitrescens</i> was isolated ^w		% Samples with viable pycnidia of <i>S. pyripitrescens</i>	
				Mean	Range	Mean	Range
2004	10-May	Crab Apple	100.0	90.0 ± 7.1 ^x	33.3-100.0	100.0 ± 0.0 a	100.0-100.0
		Fuji	50.0	...	...	33.3 ± 13.1 b	0.0-100.0
	21-June	Crab Apple	100.0	90.0 ± 5.1 a ^z	66.7-100.0	100.0 ± 0.0 a	100.0-100.0
		Fuji	70.0	46.7 ± 8.9 b	0.0-100.0	30.0 ± 7.8 b	0.0-66.7
	10-Aug	Crab Apple	100.0	56.7 ± 12.2 a	0.0-100.0	93.3 ± 4.4 a	66.7-100.0
		Fuji	57.5	20.0 ± 4.1 b	0.0-66.7	30.8 ± 5.3 b	0.0-100.0
	24-Sep	Crab Apple	100.0	73.3 ± 4.4	66.7-100.1	93.3 ± 4.4 a	66.7-100.0
		Fuji	50.0	...	...	20.0 ± 7.4 b	0.0-66.7
	3-Nov	Crab Apple	100.0	90.0 ± 5.1	66.7-100.0	96.7 ± 3.3 a	66.7-100.0
		Fuji	70.0	...	...	36.7 ± 10.5 b	0.0-100.0
2005	13-Apr	Crab Apple	100.0	76.7 ± 11.2	0.0-100.0	100.0 ± 0.0	100.0-100.0
		Fuji	...	...	...	...	...
	13-May	Crab Apple	100.0	86.7 ± 7.4 a	33.3-100.0	86.7 ± 7.4 a	33.3-100.0
		Fuji	90.0	66.7 ± 14.9 b	0.0-100.0	53.3 ± 11.3 b	0.0-100.0
	22-June	Crab Apple	100.0	70.0 ± 9.2 a	0.0-100.0	73.3 ± 8.3 a	33.3-100.0
		Fuji	60.0	25.9 ± 14.5 b	0.0-100.0	26.7 ± 8.3 b	0.0-66.7
	4-Aug	Crab Apple	100.0	83.3 ± 7.5 a	33.3-100.0	96.7 ± 3.3 a	66.7-100.0
		Fuji	60.0	45.8 ± 15.0 b	0.0-100.0	26.7 ± 8.3 b	0.0-66.7
	16-Sep	Crab Apple	100.0	93.3 ± 4.4 a	66.7-100.0	100.0 ± 0.0 a	100.0-100.0
		Fuji	70.0	16.7 ± 6.7 b	0.0-50.0	40.0 ± 10.9 b	0.0-100.0
2006	9-Nov	Crab Apple	100.0	90.0 ± 5.1	66.7-100.0	100.0 ± 0.0 a	100.0-100.0
		Fuji	70.0	...	...	50.0 ± 13.4 b	0.0-100.0
	18-Apr	Crab Apple	100.0	93.3 ± 6.7	33.3-100.0	93.3 ± 6.7	33.3-100.0
		Fuji	...	...	...	...	...
	16-May	Crab Apple	100.0	90.0 ± 7.1 a	33.3-100.0	96.7 ± 3.3 a	66.7-100.0
		Fuji	80.0	90.0 ± 6.7 a	50.0-100.0	43.3 ± 10.0 b	0.0-100.0
	20-June	Crab Apple	90.0	86.7 ± 10.2 a	0.0-100.0	90.0 ± 10.0 a	0.0-100.0
		Fuji	80.0	78.6 ± 10.7 a	33.3-100.0	46.7 ± 8.9 b	0.0-66.7
	10-Aug	Crab Apple	100.0	80.0 ± 7.4 a	33.3-100.0	100.0 ± 0.0 a	100.0-100.0
		Fuji	90.0	75.9 ± 9.7 a	33.3-100.0	56.7 ± 10.0 b	0.0-100.0
	27-Sep	Crab Apple	100.0	76.7 ± 7.1 a	33.3-100.0	93.3 ± 4.4 a	66.7-100.0
		Fuji	100.0	88.3 ± 6.1 a	50.0-100.0	76.7 ± 8.7 a	33.3-100.0
	16-Nov	Crab Apple	100.0	96.7 ± 3.3 a	66.7-100.0	96.7 ± 3.3 a	66.7-100.0
		Fuji	90.0	40.7 ± 14.2 b	0.0-100.0	66.7 ± 9.9 b	0.0-100.0

^a At each sampling time, 3 twigs with dieback or canker or fruit spurs were collected from each of 10 crabapple and 10 Fuji apple trees. Crabapple was planted as a source of pollen for apple production.

^v Viability of pycnidia and conidia was assessed by plating conidia on acidified potato dextrose agar to examine the development of *S. pyripitrescens* colonies.

^w Recovery of *S. pyripitrescens* from sampled twigs or fruit spurs by isolation from diseased tissues.

^x Values are mean and standard error based on the samples from 10 trees.

^y Isolation from the samples was not performed or samples were not taken.

^z Values followed by the same letter in the same column on the same sampling date are not significantly different according to the Student's *t* test with *P* = 0.05.

least one row apart between rows, and three to five trees apart within a row. Three twigs with dieback from each crabapple tree and three dead fruit spurs or twigs from each Fuji tree were collected at each sampling time. Isolation of *S. pyripitrescens* from diseased twigs was attempted as described above. Samples were also examined for the presence of pycnidia of *S. pyripitrescens*. If pycnidia of *S. pyripitrescens* were present on the samples, three pycnidia were then selected and crushed in sterile water, and viability of conidia was assessed by plating drops of conidia on APDA as described above. In Markel orchard, twig dieback and canker were not commonly observed on Red Delicious trees, but dead fruit spurs and dead bark were evident on the trees. Thus at each sampling time, 5 dead fruit spurs and 10 pieces of dead bark tissues from each of 10 trees were collected. Trees were selected in the same manner as described for sampling in Morris orchard. Samples were examined for the presence of pycnidia, and viability of pycnidia was assessed as described above.

Sphaeropsis rot in stored fruit resulting from infections in the orchard. Development of Sphaeropsis rot on Fuji apple fruit during cold storage resulting from natural infections of fruit by *S. pyripitrescens* in Morris orchard was monitored in 2004–2005, 2005–2006, and 2006–2007 seasons. Fruit were harvested from Morris orchard on 3 November 2004, 21 October 2005, and 31 October 2006. No fungicides were applied in this orchard. In each year, 20 boxes of fruit were harvested from the orchard, and approximately 80 fruits from two to three trees were randomly harvested for one box. The fruit were placed on sterilized fiberboard apple trays (20 fruits per tray) wrapped in perforated polyethylene bags and stored in cardboard apple boxes at 0°C in regular atmosphere. Development of Sphaeropsis rot on harvested fruit during storage was monitored monthly up to 8 months after harvest. Each fruit was examined for Sphaeropsis rot. Symptoms of stem-end rot, calyx-end rot, or skin rot were recorded. Incidence of Sphaeropsis rot was determined and expressed as percentage of the fruit with Sphaeropsis rot symptoms in the total fruit.

Sphaeropsis rot in stored Red Delicious fruit resulting from infections in Markel orchard also was assessed in 2004–2005 and 2005–2006 seasons. In this orchard, ziram is commonly applied at

2 weeks before harvest for control of postharvest diseases. In this study, selected trees were marked and were not treated with ziram. In each year, four bins of fruit (approximately 400 kg fruit per bin) were harvested from the selected trees that did not receive fungicide sprays. Fruit were harvested on 23 September 2004 and 28 September 2005 and stored at 0°C in controlled atmosphere (<1% CO₂ and 1.5% O₂) at a commercial fruit storage facility. The commercial controlled atmosphere storage room was sealed for long-term storage of the fruit and was not unsealed until May or July the following year. Thus, the fruit were assessed only once for incidence of Sphaeropsis rot after removal of fruit from storage, on 26 May 2005 for the 2004–2005 experiment and on 13 July 2006 for the 2005–2006 experiment. To assess Sphaeropsis rot, each bin of fruit was processed on a research packing line to separate decayed fruit from sound fruit. The total number of fruit from each bin was automatically counted by the machine when the fruit passed through the packing line. Decayed fruit were examined for Sphaeropsis rot, and the number of fruit with Sphaeropsis rot symptoms was recorded. If necessary, isolation from decayed tissue was performed to confirm the cause of decayed fruit, following the procedure of Kim and Xiao (5).

Seasonal survival and pycnidial production. To determine the survival and pycnidial production in infected apple twigs under orchard conditions, twig inoculation experiments were conducted in a Golden Delicious apple block in 2004–2005 and in a Fuji apple block in 2005–2006 and 2006–2007 at a research orchard located at the Washington State University Tree Fruit Research and Extension Center (WSU TFREC) in Wenatchee, WA. The isolate CLX1776 of *S. pyripitrescens* that was originally isolated from a decayed ‘Granny Smith’ apple fruit was used in the study. The isolate was stored as PDA mycelium plugs in 15% glycerol at –80°C and revived on PDA before its use.

In each year, 2-year-old apple twigs were tagged, sprayed with 70% ethanol, and allowed to dry. Each twig was wounded into the cambium using a cork borer. The wounded area of the twig was covered with a piece of cardboard with a hole (2 cm long and 0.5 cm wide) and the wound at the center of the hole, and then the exposed section was sprayed for 12 s with a commercial aerosol

Table 2. Inoculum availability of *Sphaeropsis pyripitrescens* in a commercial ‘Red Delicious’ apple orchard, Manson, Washington State

Year	Sampling date	Type of sample ^x	% Trees with viable pycnidia of <i>S. pyripitrescens</i> ^y	% Samples with viable pycnidia of <i>S. pyripitrescens</i>	
				Mean	Range
2004	10-May	Twig	0.0	0.0 ± 0.0 a ^z	0.0
		Bark	30.0	4.0 ± 2.2 a	0.0–20.0
	21-June	Twig	10.0	2.0 ± 2.0 a	0.0–20.0
		Bark	0.0	0.0 ± 0.0 a	0.0
	10-Aug	Twig	40.0	8.0 ± 3.3 a	0.0–20.0
		Bark	20.0	2.0 ± 1.3 a	0.0–10.0
2005	24-Sep	Twig	0.0	0.0 ± 0.0	0.0
		Bark	0.0	0.0 ± 0.0	0.0
	13-May	Twig	40.0	8.0 ± 3.3 a	0.0–20.0
		Bark	20.0	3.0 ± 1.5 a	0.0–10.0
	22-June	Twig	10.0	2.0 ± 2.0 a	0.0–20.0
		Bark	0.0	0.0 ± 0.0 a	0.0
	4-Aug	Twig	40.0	8.0 ± 3.3 a	0.0–20.0
		Bark	0.0	0.0 ± 0.0 b	0.0
	16-Sep	Twig	20.0	6.0 ± 4.3 a	0.0–40.0
		Bark	30.0	3.0 ± 1.5 a	0.0–10.0
2006	16-May	Twig	0.0	0.0 ± 0.0	0.0
		Bark	0.0	0.0 ± 0.0	0.0
	20-June	Twig	20.0	4.0 ± 2.7 a	0.0–20.0
		Bark	20.0	2.0 ± 1.3 a	0.0–10.0
	10-Aug	Twig	50.0	12.0 ± 4.4 a	0.0–40.0
		Bark	0.0	0.0 ± 0.0 b	0.0
	27-Sep	Twig	50.0	14.0 ± 5.2 a	0.0–40.0
		Bark	0.0	0.0 ± 0.0 b	0.0

^x At each sampling time, 5 dead fruit spurs and 10 pieces of dead bark were collected from each of 10 apple trees.

^y Viability of pycnidia and conidia was assessed by plating conidia on APDA to examine the development of *S. pyripitrescens* colonies.

^z Values are mean and standard error based on samples from 10 trees, and values followed by the same letter in the same column on the same sampling date are not significantly different according to the Student's *t* test with *P* = 0.05.

tissue freezing product (Freeze'IT; Curtin Matheson Scientific, Houston, TX) (13). The purpose of the cold injury treatment prior to inoculation was to mimic predisposition of twigs to cold weather that occurs in winter. To inoculate twigs, a 5-mm mycelium plug from a 4-day-old APDA culture of *S. pyripitrescens* was placed at each wound. The inoculation site was then covered with moistened cheesecloth and wrapped with Parafilm, which was removed 21 days after inoculation. Four trees and 18 twigs per tree of Golden Delicious apple were inoculated on 3 December 2004, and five trees and 18 twigs per tree of Fuji apple were inoculated on 2 De-

cember 2005 and 4 December 2006. Weather data were obtained from a weather station near the orchard maintained by the WSU TFREC.

To monitor the survival and pycnidia production on inoculated twigs, two twigs were removed from each tree at 1, 2, 4, 6, 8, 10, and 12 months after inoculation. Twigs were examined under a stereo-microscope for the presence of pycnidia of *S. pyripitrescens*. Any teleomorph state of a fungus, if present, was examined, and isolation from ascospores was attempted in order to establish a possible connection of *S. pyripitrescens* with the

Fig. 1. Sources of *Sphaeropsis pyripitrescens* inoculum in apple orchards: **A**, canker caused by the fungus on a Fuji apple branch; **B**, pycnidia of the fungus on a dead Fuji fruit pedicle; **C**, dieback caused by the fungus on a Fuji twig; **D**, pycnidia on a diseased twig; **E**, dieback and canker on a crabapple tree; **F**, canker on the main branch of a crabapple tree with pycnidia of the fungus on the diseased tissues; **G**, mummified Fuji fruit showing pycnidia of the fungus in the spring (May); and **H**, mummified crabapple fruit showing pycnidia of the fungus during Fuji apple harvest in late October. Scale bar in **D** = 0.25 μ m.

teleomorphic fungus. Isolation of the fungus from the margin of diseased and healthy tissues of inoculated twigs was performed to determine whether the fungus survived over a period of time after inoculation.

Data analysis. For the data from monitoring inoculum availability, a *t* test was performed with SAS PROC TTEST (Version 9.2, SAS Institute, Cary, NC) to compare the differences in percent samples from which *S. pyriputrescens* was recovered by isolation from diseased tissues and percent samples with viable pycnidia of the fungus between crabapple and Fuji apple samples. A *t* test was also performed to compare the differences in percent samples with viable pycnidia of the fungus between the bark and fruit spur samples of Red Delicious (2 levels, diseased and nondiseased, for tissue type). To compare percentage of Sphaeropsis rot originating from different infection sites of Fuji fruit naturally infected by *S. pyriputrescens* in the Morris orchard, analysis of variance with SAS PROC GLM was performed with infection site (3 sites) as independent variable, and mean separation was performed using Fisher's least significant difference (LSD, $P = 0.05$). The data from the 3-year study on seasonal survival and pycnidial production on inoculated twigs were subjected to analysis of variance using SAS PROC GLM, with year as block and sampling time (7 times) as independent variable. Means of the 3-year combined data were presented for ease of presentation. All data in percentage were arcsine-square-root-transformed prior to analysis.

Results

Sources of inoculum. *S. pyriputrescens* pycnidia were observed on diseased twigs, dead fruit spurs and bark, and mummified fruit on both apple and crabapple trees (Fig. 1). Pycnidia of *S. pyriputrescens* were observed on 57 and 27% of the sampled mummies in May 2005 and May 2006, respectively (Fig. 1G). At the time of the Fuji harvest in 2004, *S. pyriputrescens* was isolated from decayed crabapple fruit from 13 of the 14 crabapple trees (93%) sampled, and 3% of the decayed crabapple fruit caused by *S. pyriputrescens* also had pycnidia of the fungus present on the fruit (Fig. 1H).

Availability of viable inoculum in the orchard. In Morris orchard, viable pycnidia of *S. pyriputrescens* were observed at all

sampling times during the 3-year study (Table 1). All crabapple trees, except one tree sampled on 20 June 2006, harbored viable pycnidia of *S. pyriputrescens* during the 3-year study, and 50 to 100% of the sampled Fuji trees had viable pycnidia of the fungus (Table 1). *S. pyriputrescens* was isolated from 56.7 to 93.3% of the diseased twigs of crabapple trees and 16.7 to 90% of the Fuji twigs or fruit spurs over the 3-year study. Viable pycnidia were observed on 73.3 to 100% of the crabapple twig samples and 20 to 76.7% of the Fuji twigs or fruit spur samples over the 3-year study. At the majority of sampling times, the percentages of sampled twigs from which *S. pyriputrescens* was isolated and percentages of sampled twigs from which viable pycnidia were observed from crabapple were significantly higher than those from Fuji apple (Table 1).

In Markel orchard, viable pycnidia of *S. pyriputrescens* were observed on 10 to 50% of the sampled Red Delicious trees during the 3-year study, except the samples taken on 24 September 2004 and 16 May 2006 (Table 2). Viable pycnidia were observed on 0 to 14% of the sampled twigs and 0 to 4% of the sampled bark.

Sphaeropsis rot during storage. During storage, Sphaeropsis rot resulting from natural infections by *S. pyriputrescens* in the orchard was observed on Fuji apples harvested from the Morris orchard in all three seasons (Fig. 2). Symptoms were first observed 1 to 2 months after harvest, and incidence of Sphaeropsis rot increased as the storage period extended up to about 5 to 6 months after harvest. By 8 months after harvest, 24, 4.3, and 39.6% of the fruit developed Sphaeropsis rot in 2004–2005, 2005–2006, and 2006–2007 seasons, respectively (Fig. 2). Analysis of variance (ANOVA) indicated that there were no significant differences ($P > 0.05$) in percent Sphaeropsis rot originating from stem and calyx infections in 2005–2006 and 2006–2007 seasons, but there were more calyx-end rot than stem-end rot in 2004–2005 season (Fig. 3). Stem-end Sphaeropsis rot and calyx-end Sphaeropsis rot were the most common symptoms on the fruit, accounting for 19.6 to 63.6% and 36.4 to 78.1% of the rots, respectively, whereas Sphaeropsis rot originating from wound infections on the fruit skin accounted for 0 to 4.1% (Fig. 3).

For the Red Delicious fruit harvested from Markel orchard, 4.3% of the fruit were decayed by *S. pyriputrescens* 8 months after harvest in 2004–2005, and 15.1% of the fruit were decayed by *S.*

Fig. 2. Development of Sphaeropsis rot on Fuji apple fruit during cold storage resulting from natural infections of fruit caused by *Sphaeropsis pyriputrescens* in the Morris orchard from 2004 to 2007. Twenty boxes each with approximately 80 fruit were harvested in late October or early November, stored at 0°C, and monitored monthly for Sphaeropsis rot for up to 8 months after harvest. Incidence of Sphaeropsis rot was expressed as percentage of the fruit with Sphaeropsis rot symptoms in the total fruit.

Fig. 3. Percentages of Sphaeropsis rot originating from natural infections at the stem, calyx, or skin (lenticel infection or wound infection) among the decayed fruit with Sphaeropsis rot symptoms of Fuji apple fruit stored at 0°C for 8 months after harvest conducted from 2004 to 2007. In each year, 20 boxes each with approximately 80 fruit were harvested from Morris orchard and stored for Sphaeropsis rot development. Bars marked with the same lower- or uppercase letter within the same season are not significantly different at $P = 0.05$ according to Fisher's protected least significant difference.

pyriputrescens 10 months after harvest in 2005–2006. In both seasons, the majority of Sphaeropsis rot-decayed fruit exhibited stem-end rot or calyx-end rot symptoms (actual percentage by infection sites was not recorded for the fruit from this orchard).

Seasonal survival and pycnidial formation on inoculated twigs. *S. pyriputrescens* was recovered from diseased tissues of inoculated twigs at all sampling times up to 12 months after inoculation (Fig. 4). ANOVA indicated that there were no significant differences among 3 years in recovery (%) from inoculated twigs ($P > 0.1854$) and percent inoculated twigs yielding pycnidia ($P > 0.145$), but significant difference ($P < 0.0157$) among 3 years was observed for percent inoculated twigs yielding viable pycnidia. On average of 3-year combined data, the fungus was recovered from 82.5 to 100% of the twigs. No pycnidia of *S. pyriputrescens* were observed on inoculated twigs at 1 and 2 months after inoculation, except that immature pycnidia were observed on 20% of the inoculated twigs 2 months after inoculation in 2004–2005. During the samplings at 4 to 12 months after inoculation, viable pycnidia of the fungus were observed on an average of 81.7 to 93.3% of the twigs based on 3-year combined data (Fig. 4).

Discussion

Twigs with dieback and cankers, dead fruit spurs and bark, and mummified fruit of apple and/or crabapple were sources of *S. pyriputrescens* inoculum in apple orchards. Pycnidia of *S. pyriputrescens* containing viable conidia were available throughout the apple fruit-growing season from April to November in north-central Washington State, and *S. pyriputrescens* survived as mycelium in diseased twigs and was able to produce pycnidia on diseased twigs throughout the year in the region. The results suggest that availability of *S. pyriputrescens* inoculum is unlikely a limiting factor for infection of apple fruit in the orchard leading to Sphaeropsis rot during storage.

It appeared that Manchurian crabapple was highly susceptible to *S. pyriputrescens*, as the incidence of twig dieback caused by the fungus was high in Morris orchard. Because crabapple trees and apple trees were planted next to each other, pycnidia on diseased crabapple trees could be spread onto adjacent apple fruit and serve as inoculum for infection of fruit. In Morris orchard, both diseased

crabapple and apple trees could serve as sources of inoculum of *S. pyriputrescens*, as *S. pyriputrescens* pycnidia were observed during the fruit growing season on both crabapple and apple trees. However, in Markle orchard where there were very few crabapple trees, pycnidia of the fungus were observed on apple trees, indicating that Sphaeropsis rot can also be a problem in apple orchards where few crabapples are planted.

S. pyriputrescens grows at temperatures ranging from -3 to 25°C and does not actively grow at 30°C but can survive this temperature (7). The average temperatures in December and January (coldest months) are -1°C in Wenatchee and -1.5°C in Manson; the average temperatures in July and August (hottest months) are 24°C in Wenatchee and 23°C in Manson (www.weather.com). These temperatures are within the range suitable for mycelial growth of the fungus (7). The average temperatures during the 3-year period of this study were within the normal average temperatures for the Wenatchee and Manson areas. In this study, *S. pyriputrescens* was recovered from diseased tissues of inoculated twigs throughout the year during the 3-year study. Our results suggest that *S. pyriputrescens* is well adapted to the semiarid climate in central Washington State. Recently, *S. pyriputrescens* has also been reported to cause Sphaeropsis rot in apple fruit in New York State, but it appeared that it was a minor problem (3). The presence of *S. pyriputrescens* outside the U.S. Pacific Northwest suggests the fungus may have the ability to adapt to climates different than that of central Washington.

Examination of naturally infected twigs and inoculated twigs over the 3 years in this study showed no presence of a teleomorph state associated with the diseased samples, and that the presence of *S. pyriputrescens* pycnidia was observed at all sampling times, suggesting that pycnidia are the major type, if not the only type, of inoculum responsible for infection of apple fruit in the orchards leading to Sphaeropsis rot during storage. The availability of viable pycnidia throughout the sampling periods suggests that pycnidia can survive for a long period of time under orchard conditions in north-central Washington State.

In a separate study involving inoculation of Fuji, Red Delicious, and Golden Delicious apple fruit with *S. pyriputrescens* at various times during the fruit growing season in the orchard, it was found that infections of apple fruit by *S. pyriputrescens* can occur at any inoculation time from 3 weeks after petal fall to near harvest (4), suggesting that if viable inoculum is available and conducive conditions are met, infections of apple fruit by *S. pyriputrescens* can occur at various stage of fruit development during the fruit growing season. In the present study, apple fruit at harvest from both Morris and Markle orchards, in which viable inoculum of *S. pyriputrescens* was present during the fruit growing season, did not show rot symptoms, but Sphaeropsis rot developed on these fruit during cold storage after harvest. These results together indicate that Sphaeropsis rot is an orchard-originated postharvest fruit rot disease resulting from latent infections of fruit by *S. pyriputrescens* in the orchard. Our results also suggest that the epidemiology of Sphaeropsis rot in apple caused by *S. pyriputrescens* is similar to that of Phacidiopycnis rot in pear fruit caused by *Potrebniomyces pyri* (anamorph *Phacidiopycnis piri*) and that of bull's-eye rot in apple and pear fruit caused by *Neofabraea perennans* and other *Neofabraea* spp. Both *P. pyri* and *N. perennans* are canker-causing pathogens to apple/pear trees, survive on the trees, and cause latent infections in the fruit in the orchard leading to fruit rots during storage (1,2,8,14,17,19). Like *S. pyriputrescens*, *P. pyri* also was recently recognized in the United States and Phacidiopycnis rot is a major postharvest fruit rot disease of d'Anjou pears grown in Washington State (16). The recent reports of *S. pyriputrescens* in apple and *P. pyri* in pear in the U.S. Pacific Northwest indicated that control of postharvest diseases of pome fruits in the U.S. Pacific Northwest should target not only fruit rots resulting from common wound-invading postharvest pathogens such as *Penicillium expansum* but also those from latent fungal pathogens such as *Neofabraea* spp. and the recently reported *S. pyriputrescens* and *P. pyri*.

Fig. 4. Recovery of *Sphaeropsis pyriputrescens* by isolation from inoculated apple twigs and percentages of inoculated twigs with the presence of pycnidia and viable pycnidia of the fungus 1 to 12 months after inoculation. Experiments were conducted from 2004 to 2006. Twigs were inoculated in early December each year. Two inoculated twigs were removed from each of 4 or 5 trees at each sampling time for isolation of the fungus and examination for the presence of pycnidia and viability of conidia of *S. pyriputrescens*. Values are the means of 3-year combined data, and error bars are standard errors of means.

While viable inoculum of *S. pyripitrescens* was available during the fruit growing season in both orchards, the incidence of Sphaeropsis rot in stored fruit resulting from natural infections in the orchards varied from year to year, suggesting that other factors such as micro-environmental conditions and fruit susceptibility at different growth stages in the orchard may play a more important role than inoculum availability in initiating infection of apple fruit. As free moisture is needed for pycnidia of *S. pyripitrescens* to rupture and for conidia to germinate (6), irrigation and rain likely play an important role in the spread of this pathogen and initiation of infection. In Washington State, irrigation in apple orchards is usually supplied with under tree sprinklers. But in recent years, over-tree evaporative cooling is often used in modern apple orchards to prevent sunburn on the fruit in summer because apple fruit on the dwarfing trees in these orchards could be directly exposed to sunlight in the summer leading to sunburn on the fruit (12). Over-tree evaporative cooling may run for several hours, facilitating the spread of conidia of *S. pyripitrescens* from infected trees to adjacent trees and creating a long period of wetness duration on the fruit conducive to infection by the fungus. Minimizing the use of over-tree evaporative cooling or use of alternatives to evaporative cooling, such as spraying the fruit with clay or film-forming products (11), may avoid creating conditions favorable for spread and infection of apple fruit by *S. pyripitrescens*. Future research is needed to determine relationships between fruit infections at different growth stages and environmental variables such as temperature and wetness duration on the fruit. Such information would help us further understand conditions required for fruit infections and develop relevant measures for disease control.

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