Control of Sphaeropsis Rot in Stored Apple Fruit Caused by *Sphaeropsis pyriputrescens* with Postharvest Fungicides

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Abstract

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Sphaeropsis rot caused by *Sphaeropsis pyriputrescens* is a recently reported postharvest fruit rot disease of apple grown in Washington State. The objective of this study was to develop chemical-based mitigation measures for Sphaeropsis rot in stored apple fruit. To determine in vitro sensitivity of *S. pyriputrescens* to the three registered postharvest fungicides thiabendazole, fludioxonil, and pyrimethanil, 30 isolates of *S. pyriputrescens* obtained from various sources were tested for mycelial growth and conidial germination on fungicide-amended media. Golden Delicious apple fruit were inoculated with the pathogen in the orchard at 2 or 5 weeks before harvest. After harvest, fruit were either nontreated or dipped in thiabendazole, fludioxonil, or pyrimethanil solutions, stored at 0°C, and monitored for decay development for up to 9 months after harvest. The mean effective concentration of a

fungicide that inhibits mycelial growth or spore germination by 50% relative to the nonamended control (EC₅₀) values of thiabendazole, fludioxonil, and pyrimethanil on mycelial growth were 0.791, 0.0005, and 2.829 µg/ml, respectively. Fludioxonil and pyrimethanil also were effective in inhibiting conidial germination of the fungus with EC₅₀ values of 0.02 µg/ml for fludioxonil and 5.626 µg/ml for pyrimethanil. All three postharvest fungicides applied at label rates immediately after harvest were equally effective in controlling Sphaeropsis rot in stored apple fruit, reducing disease incidence by 92 to 100% compared with the nontreated control. The results indicated that Sphaeropsis rot may be effectively controlled by the currently registered postharvest fungicides thiabendazole, fludioxonil, and pyrimethanil.

Washington State is the leading apple-producing state in the United States. In 2009, apple production in Washington accounted for approximately 54% of the U.S. total, with production values of over \$1.2 billion (19,20). Approximately 30% of the Washington-grown apple fruit are destined for export. At harvest, fruit are hand picked and placed into wooden or plastic bins which are then transported to storage and packing facilities. Fruit are typically stored in either regular or controlled atmosphere (1 to 2% O₂ and 0.5% CO₂) at -1 to 3°C, depending on cultivar (12). Fruit may be stored in field bins for an extended period of time because the large volume of fruit is packed and marketed over a period of 10 to 12 months following harvest. However, postharvest fruit rot diseases can be a limiting factor for storage of apple fruit. If left unmanaged, postharvest diseases can result in significant economic losses of apple fruit in storage, during transit, or in the market. Furthermore, for the fruit destined for export, postharvest rots present in the shipments may pose quarantine concerns if the diseases are listed as quarantine targets in the destination countries, potentially leading to more economic losses.

Several postharvest diseases commonly occur on apple in Washington State (8). Control of postharvest diseases in apple has largely relied on the postharvest use of fungicides, commonly applied as a prestorage drench (2,24). In the United States, four fungicides are currently available for postharvest use on apple: captan, thiabendazole, fludioxonil, and pyrimethanil. However, captan is not commonly used by fruit packers due, in part, to resi-

due concerns in some overseas markets. Prior to 2005, thiabendazole was the most commonly used postharvest fungicide on apple but resistance to thiabendazole in *Penicillium expansum*, the cause of blue mold, often results in control failures (3,11). Fludioxonil and pyrimethanil, belonging to the chemical classes of phenylpyrrole and anilinopyrimidine, respectively (4), were registered in late 2004 in the United States for postharvest use on apple and pear. According to the United States Environmental Protection Agency classification, fludioxonil and pyrimethanil are considered reduced-risk fungicides, which pose a low risk to humans and the environment (18). Fludioxonil and pyrimethanil are highly effective in controlling gray mold caused by *Botrytis cinerea* and blue mold, which are major postharvest diseases affecting apple (3,11,15), and these two fungicides are being increasingly used by apple packers in Washington as alternatives to thiabendazole for control of gray mold and blue mold (24).

Sphaeropsis rot, caused by *Sphaeropsis pyriputrescens* C.L. Xiao & J.D. Rogers, is a recently described postharvest fruit rot disease of apple and pear, initially reported on the latter host (8,25). Later, it was observed that the disease caused even more serious problems on apple (26). In one instance where no fungicides had been applied to 'Red Delicious' fruit, 24% of the fruit were decayed by *S. pyriputrescens* in storage (26). A recent survey for postharvest diseases of apple conducted on Red Delicious, 'Fuji', and 'Golden Delicious' in Washington State indicated that, in addition to gray mold and blue mold, Sphaeropsis rot was a major postharvest disease of apple, accounting for approximately 17% of the total rots, and that *S. pyriputrescens* was prevalent in all major apple-production counties in central Washington, which is the major apple-producing areas in Washington (8).

S. pyriputrescens also is the cause of a canker and twig dieback disease of apple and crabapple trees (22). Viable pycnidia of the fungus on infected tissues of apple trees can be found throughout the fruit growing season (C. L. Xiao, unpublished data). Infections of apple fruit by *S. pyriputrescens* occur in the orchard but remain latent, and Sphaeropsis rot symptoms develop in storage (7,26). Although the basic biology of *S. pyriputrescens* has been described (9,10), little information is available on the control of Sphaeropsis

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rot in stored apple fruit. Because *Sphaeropsis* rot is a major component of postharvest fruit rots in apple grown in Washington, information is needed as to whether the currently labeled postharvest fungicides are effective for controlling this disease in stored apple fruit. Moreover, currently, *S. pyripitrescens* has been reported only in North America, and it is unknown whether it is also distributed in other apple-production regions in the world (23). Consequently, *S. pyripitrescens* has been listed as a quarantine pathogen by some other countries such as China; thus, mitigation measures for *Sphaeropsis* rot are needed in order for the apple industry in the region to export apple fruit to these markets in compliance with phytosanitary requirements.

The objectives of this study were to assess the sensitivity of mycelial growth and conidial germination of *S. pyripitrescens* to the three postharvest fungicides thiabendazole, fludioxonil, and pyrimethanil registered for use on apple, and to evaluate the effectiveness of the three postharvest fungicides for controlling *Sphaeropsis* rot in storage.

Materials and Methods

Fungal isolates. Twenty isolates of *S. pyripitrescens* recovered from decayed apple fruit during a survey for postharvest diseases conducted from 2003 to 2005 (8), five isolates recovered from diseased twigs of apple trees, and five isolates from diseased crab-apple twigs were used in this study. Isolation of the fungus from decayed fruit and diseased twigs was performed on acidified potato dextrose agar (APDA; Difco Laboratories, Detroit) (4 ml of a 25% solution of lactic acid per liter of medium) following the procedures of Kim and Xiao (8) and Xiao and Boal (22), respectively. Fungi that grew on APDA were transferred onto fresh APDA. Pure cultures were then grown on PDA and stored in 15% glycerol at -80°C . Identification of *S. pyripitrescens* was based upon the descriptions of the fungus by Xiao and Rogers (25). Prior to the experiment, all isolates used in this study were single-conidium cultured. Pycnidia from PDA cultures incubated for 3 weeks under a cycle of 12 h of light and 12 h of darkness (10 Wm^{-2}) at 20°C were crushed in sterile water on glass slides, and conidia were then streaked on PDA and incubated overnight at 20°C . Individual germinated conidia were selected under a microscope to transfer onto PDA to establish single-conidium cultures.

Sensitivity of mycelial growth and conidial germination of *S. pyripitrescens*. Technical-grade thiabendazole (a.i. 99%; Sigma-Aldrich, St. Louis), fludioxonil (a.i. 93%; Syngenta Inc., Switzerland), and pyrimethanil (a.i. 96%; Janssen Pharmaceutical N.V., Belgium) were used for in vitro sensitivity tests. Stock solutions of thiabendazole and pyrimethanil were made in dimethyl sulfoxide, and stock solutions of fludioxonil were made in acetone. Thiabendazole and fludioxonil were added into PDA to produce the following concentrations: thiabendazole at 0, 0.01, 0.1, 0.25, 0.5, 1, 2.5, 10, and 50 $\mu\text{g/ml}$ and fludioxonil at 0, 0.0001, 0.0005, 0.001, 0.005, 0.01, 0.05, and 0.1 $\mu\text{g/ml}$. Sensitivity to pyrimethanil was tested on an L-asparagine-based agar medium (6) amended with pyrimethanil at 0, 0.05, 0.1, 0.25, 0.5, 1, 5, 10, 25, and 50 $\mu\text{g/ml}$. The same amount of solvent was added into the nonamended control plates.

For the mycelial growth assay, isolates were grown on PDA for 3 days at 20°C in the dark. A 5-mm-diameter plug of mycelia was removed from the margin of actively growing colonies with a sterile cork borer, placed inversely at the center of each plate, and incubated at 20°C in the dark for 3 days. Colony diameters were measured at two perpendicular directions. There were two replicate plates for each fungicide concentration for each isolate, and the experiment was performed twice.

For the conidial germination assay, isolates retrieved from -80°C were initially grown on PDA for 3 weeks under a cycle of 12 h of light and 12 h of darkness (10 Wm^{-2}) at 20°C . A previous study indicated that oatmeal agar (60 g of finely ground iron- and zinc-fortified oatmeal [Gerber, Fremont, MI] and 15 g of agar in 1 liter of deionized water, autoclaved at 121°C for 90 min) is more suitable than PDA for production of pycnidia of *S. pyripitrescens*

(10). To augment pycnidial production, conidia from oozing pycnidia from the PDA cultures were streaked on oatmeal agar, sealed with Parafilm, and incubated at 20°C under the same light-and-darkness regime for 3 weeks (10). Each plate was flooded with 20 ml of sterile deionized water and pycnidia were scrapped off the surface of the medium. The resulting conidial suspension was filtered through two layers of cheesecloth and the final concentration was adjusted to 2×10^5 conidia/ml with a hemacytometer. Conidial suspension (30 μl) was spread with a sterile glass rod on each plate of fungicide-amended medium and incubated for 24 h at 20°C in the dark. Germination efficiency was estimated by examining 200 conidia/plate. A conidium was considered germinated if the germ tube was at least one-half the length of the conidium. Percent inhibition of each isolate at each fungicide concentration relative to the nonamended control was calculated. Two replicate plates of each fungicide concentration were used for each isolate. The experiment was conducted twice for all isolates. Thiabendazole is a benzimidazole fungicide. Benzimidazole fungicides primarily target mycelial growth and a mycelial growth assay is recommended for sensitivity tests in fungal pathogens (16). Thus, thiabendazole was not tested for activity on conidial germination of *S. pyripitrescens*.

Efficacy of postharvest fungicides in controlling *Sphaeropsis* rot in storage. Isolate CLX2380 recovered from a decayed apple fruit was used in this study. Conidial suspensions for fruit inoculation in the orchard were prepared as described above. The final concentration was adjusted to 5×10^5 conidia/ml with a hemacytometer in 0.001% Tween 20. Viability of conidia in the inoculum was confirmed by examining germination rate of conidia on PDA after 15 h of incubation at 20°C as described above, and over 92% of the conidia were able to germinate.

The experiments of fruit inoculation were conducted in 2006 and 2007 in a research orchard of Golden Delicious trees (planted in 1985) located at the Washington State University Tree Fruit Research and Extension Center, Wenatchee. Insects and weeds were controlled following the recommendations commonly used for commercial apple production in the region (17). In each year, one application of trifloxystrobin (Flint 50W at 175 g/ha with a spray volume of 1,871 liters/ha) was made using a handgun sprayer in late April and followed by another one in early June to control powdery mildew on the foliage. No fungicides were used in this orchard after early June. The reason that only trifloxystrobin was used to control powdery mildew was that it was least inhibitory to *S. pyripitrescens* in in vitro sensitivity tests among the fungicides commonly used in the region for control of powdery mildew in apple (Y. K. Kim and C. L. Xiao, unpublished data). Thus, the early-season applications of trifloxystrobin for powdery mildew control likely had no or limited effects on infection of fruit by *S. pyripitrescens* if residues of trifloxystrobin were still present in fruit at the time of inoculation by *S. pyripitrescens*.

To determine whether postharvest fungicide treatments were effective in controlling *Sphaeropsis* rot in stored fruit originating from latent infections by *S. pyripitrescens* throughout the growing season, fruit were inoculated with the conidial suspension at either 2 or 5 weeks before harvest in both 2006 to 2007. At each inoculation time, four treatments (fruit inoculated in the orchard without any postharvest fungicide treatments or fruit inoculated in the orchard and treated with thiabendazole, fludioxonil, or pyrimethanil after harvest) were arranged as a randomized complete block design with four replicates per treatment. There were two trees per replicate for each treatment, and 40 fruit per replicate were randomly selected on the two trees. The fruit were inoculated with the conidial suspension using a hand sprayer until runoff. The fruit were then covered with moistened plastic bags to maintain high humidity and white paper bags to prevent heat from sunlight in the morning reaching the fruit. Inoculations were performed near sunset to avoid high temperatures affecting viability of the conidia. The bags were removed approximately 15 h after inoculation.

Fruit were harvested 2 or 5 weeks after inoculation in both 2006 and 2007 and immediately transported into the laboratory. Depend-

ing on treatments, fruit were either not treated with any postharvest fungicides or dipped for 60 s in one of the three postharvest fungicide solutions: thiabendazole as Mertect 340F (1.25 ml/liter; Syngenta Crop Protection, Greensboro, NC), fludioxonil as Scholar 50WP (0.6 g/liter) in 2006 and Scholar 230SC (0.94 ml/liter) in 2007 (Syngenta Crop Protection), and pyrimethanil as Penbotec 400SC (1.25 ml/liter; Janssen Pharmaceutical N.V.). Fruit were allowed to dry and then transferred onto sterilized fiberboard apple trays wrapped in perforated polyethylene bags and stored in cardboard apple boxes at 0°C. Decay development on the fruit, infection sites (stem or calyx) on the fruit, and incidence of Sphaeropsis rot (percentage of fruit with the symptoms) were recorded monthly after harvest until 9 months after harvest.

Data analysis. The effective concentration of a fungicide that inhibits mycelial growth or spore germination by 50% relative to the nonamended control (EC_{50}) was calculated for each isolate and each fungicide. The EC_{50} values were calculated by regression of the percent relative growth against the $\log_{10}$ concentrations of thiabendazole, fludioxonil, or pyrimethanil using SAS PROC REG (version 9.1; SAS Institute, Cary, NC). Experiments to test in vitro fungicide sensitivity were performed twice for all isolates. An *F* test for equality of variances was conducted to determine whether the two experimental replicates could be combined. There was no significant ($P > 0.05$) difference between runs or run-isolate interactions; thus, pooled data were presented.

Analysis of variance was performed with SAS PROC GLM (version 9.1; SAS Institute) to determine whether incidence of Sphaeropsis rot was significantly different among the postharvest fungicide treatments. Means were separated by Fisher's protected least significant difference ($P = 0.05$). All data in percentage were arcsine-transformed prior to analysis (5).

Results

Sensitivity of mycelial growth and conidial germination of *S. pyripitrescens*. All three fungicides were effective in inhibiting mycelial growth of *S. pyripitrescens* (Table 1). EC_{50} values of thiabendazole for mycelial growth ranged from 0.612 to 1.335 $\mu\text{g/ml}$. Fludioxonil was highly effective in inhibiting mycelial growth, with EC_{50} values ranging from 0.0003 to 0.0008 $\mu\text{g/ml}$. EC_{50} values of pyrimethanil for mycelial growth ranged from 0.192 to 5.749 $\mu\text{g/ml}$.

Fludioxonil and pyrimethanil were also effective in inhibiting conidial germination of *S. pyripitrescens*, with EC_{50} values ranging from 0.013 to 0.053 $\mu\text{g/ml}$ for fludioxonil and from 1.524 to 27.508 $\mu\text{g/ml}$ for pyrimethanil (Table 1).

Efficacy of postharvest fungicides for controlling Sphaeropsis rot in storage. Stem-end rot and calyx-end rot were the two most common symptoms of Sphaeropsis rot on inoculated fruit during storage. For the fruit inoculated with *S. pyripitrescens* 5

weeks before harvest, 58 and 75% of the fruit were affected by *S. pyripitrescens* following 9 months in storage after harvest in the 2006–07 and 2007–08 seasons, respectively (Fig. 1). When applied as a postharvest treatment immediately after harvest, all three fungicides were highly effective in controlling Sphaeropsis rot, reducing Sphaeropsis rot incidence by 92 to 100% compared with the nontreated control (Fig. 1). No Sphaeropsis rot was observed on the fruit treated with pyrimethanil. Although Sphaeropsis rot was present in some fruit treated with either thiabendazole or fludioxonil, the incidence of Sphaeropsis rot was very low (3 to 6%) and there was no significant difference in the incidence of

Fig. 1. Effectiveness of thiabendazole (TBZ), fludioxonil (FLU), or pyrimethanil (PYR) as a postharvest treatment applied immediately after harvest in controlling Sphaeropsis rot (stem- and calyx-end rot) on stored 'Golden Delicious' apple in the 2006–07 and 2007–08 seasons. Fruit were inoculated 5 weeks before harvest in the orchard with the conidial suspension of *Sphaeropsis pyripitrescens*, harvested, treated with postharvest fungicides, stored at 0°C, and monitored for decay development for up to 9 months after harvest. Columns within the same pattern group marked with the same letter are not significantly different at $P = 0.05$ according to Fisher's protected least significant difference.

Table 1. Mean and range of the effective concentration of a fungicide that inhibits mycelial growth or spore germination by 50% relative to the nonamended control (EC_{50}) of thiabendazole (TBZ), fludioxonil, and pyrimethanil on mycelial growth and conidial germination of *Sphaeropsis pyripitrescens* isolates obtained from different sources

Host	Treatment ^b	Isolates ^c	Assay ^d	EC_{50} ($\mu\text{g/ml}$) ^a					
				Thiabendazole		Fludioxonil		Pyrimethanil	
				Mean	Range	Mean	Range	Mean	Range
Apple fruit	TBZ	10	Myc	0.807	0.619–1.163	0.0005	0.0004–0.0006	1.671	0.192–4.169
	...	...	Con	–	–	0.021	0.017–0.031	7.13	1.524–27.508
Apple fruit	Non	10	Myc	0.881	0.71–1.335	0.0005	0.0004–0.0006	3.014	0.59–5.749
	...	...	Con	–	–	0.023	0.016–0.053	6.053	1.588–12.003
Apple tree	Non	5	Myc	0.695	0.614–0.861	0.0004	0.0003–0.0005	3.609	0.983–5.263
	...	...	Con	–	–	0.018	0.013–0.021	3.9	2.631–7.073
Crabapple tree	Non	5	Myc	0.78	0.612–0.967	0.0005	0.0003–0.0008	3.02	0.582–4.931
	...	...	Con	–	–	0.019	0.016–0.022	5.422	2.247–15.13

^a Values are the means of pooled data from the two runs of the experiments; – indicates that TBZ was not tested for activity on conidial germination because benzimidazole fungicides primarily target mycelial growth and a mycelial growth assay is recommended for sensitivity tests in fungal pathogens (16).

^b Postharvest treatment: TBZ = TBZ-drenched and Non = not drenched.

^c Number of isolates.

^d Myc = mycelial growth and Con = conidial germination.

Sphaeropsis rot between pyrimethanil-treated fruit and those treated with either thiabendazole or fludioxonil (Fig. 1).

For the fruit inoculated 2 weeks before harvest, 44 and 73% had Sphaeropsis rot in storage 9 months after harvest in the 2006–07 and 2007–08 seasons, respectively (Fig. 2). Similarly to those observed on the fruit inoculated 5 weeks before harvest, the three fungicides were highly effective in controlling Sphaeropsis rot, reducing Sphaeropsis rot incidence by 94 to 100% compared with the nontreated control. There was no significant difference in efficacy among the three fungicides (Fig. 2).

Discussion

In this study, the 30 isolates used in in vitro fungicide sensitivity tests represented the pathogen recovered from apple or crabapple from different apple-production districts in central Washington State. These isolates had not been exposed to fludioxonil or anilino-pyrimidine fungicides cyprodinil and pyrimethanil because these fungicides either are not registered for use in apple orchards or had not been used in the orchards or on the fruit from which the isolates were obtained. Thus, the in vitro sensitivity data can serve as the baseline sensitivity of *S. pyriputrescens* to fludioxonil and pyrimethanil.

Thiabendazole belongs to the group of methyl benzimidazole carbamate (MBC) fungicides and has been a common postharvest fungicide for use on apple for control of postharvest diseases since the early 1970s (2,24). Benzimidazole resistance is now common in *P. expansum* and also is present in *B. cinerea* populations collected from apple (14,27). Some of the isolates of *S. pyriputrescens* tested in this study had been exposed to MBCs because they were obtained from thiabendazole-drenched fruit. Some isolates recovered from diseased apple and crabapple trees might have also been exposed to MBCs because thiophanate-methyl may be used in the

orchards as a preharvest spray for postharvest disease management. However, all these isolates responded to thiabendazole similarly and were not able to actively grow on the medium amended with thiabendazole at 10 µg/ml, and only a few isolates had very limited growth on the medium amended with thiabendazole at 2.5 µg/ml. A thiabendazole concentration of 5 and 10 µg/ml has been used to discriminate thiabendazole resistance in *P. expansum* and *B. cinerea*, respectively (14,16). The EC₅₀ values of thiabendazole for *S. pyriputrescens* were similar to those for *B. cinerea* and *P. expansum* (11,27). Thus, it appears that all 30 isolates tested in this study can be classified as thiabendazole-sensitive phenotypes.

Because of the nature of the target site, MBCs have a high risk for the development of resistance in fungal pathogens (4). However, inherent characteristics of both pathogen and fungicide contribute to the development of resistant populations of a fungal pathogen to a fungicide (1). Unlike *B. cinerea* and *P. expansum*, which have short lifecycles and abundant reproduction and are considered high-risk pathogens in the development of fungicide resistance (1), *S. pyriputrescens* is a pycnidial fungus and its capacity for sporulation during a given season is likely to be lower based on its biology (10). Because of the biological nature of the pycnidial fungus *S. pyriputrescens* and the fact that the use of MBCs in apple orchards is limited (one application of thiophanate-methyl per year may be used in the orchards), it is likely that risk for development of thiabendazole-resistant populations in *S. pyriputrescens* is low under the current production system.

In our study, isolates exhibited a narrow range of sensitivity to fludioxonil, and there was only a three- and fourfold difference in EC₅₀ values between the least and the most sensitive isolates for mycelial growth and conidial germination, respectively; whereas, for pyrimethanil, the isolates showed a great range of sensitivity, with an up to 30- and 18-fold difference in EC₅₀ values between the least and the most sensitive isolates for mycelial growth and conidial germination, respectively. The existence of a great range of sensitivity to pyrimethanil may indicate that, although these isolates had not been exposed to pyrimethanil, the pathogen population consisted of phenotypes varying in sensitivity to pyrimethanil. However, EC₅₀ values of pyrimethanil for all isolates, particularly on mycelial growth, were much lower than the recommended label rate 500 µg/ml, suggesting that pyrimethanil at label rate also is likely able to control the least-sensitive isolate (EC₅₀ of pyrimethanil for mycelial growth = 5.749 µg/ml), similar to the efficacy for the isolate CLX2380 (EC₅₀ for mycelial growth = 2.919 µg/ml) used in the control experiment conducted on apple fruit in this study.

Currently, no other phenylpyrrole fungicides are registered in the United States for use in apple orchards; thus, the development of resistance to fludioxonil in the pathogen populations in the orchard is unlikely. However, anilino-pyrimidine fungicides cyprodinil and pyrimethanil can be used in orchards for control of apple scab and other diseases (13,17). Because only a limited number of postharvest fungicides are available and pyrimethanil is a key postharvest fungicide for use on apple, in order to avoid or minimize the development of resistance to pyrimethanil in *S. pyriputrescens* and other major decay-causing pathogens such as *B. cinerea* and *P. expansum* in apple orchards and to retain the efficacy of the postharvest use of pyrimethanil for control of these pathogens in stored apple fruit, the use of anilino-pyrimidine fungicides in apple orchards may need to be limited or avoided because many other fungicides can be used in apple orchards for control of apple scab and other preharvest diseases in the region (13,17).

On both fruit inoculated 2 or 5 weeks before harvest, the incidence of Sphaeropsis rot in storage was higher in the 2007–08 season than in the 2006–07 season. Differences in environmental conditions during the period from inoculation to harvest between the 2 years may contribute to varying incidence of infections of fruit by the pathogen and, thus, the incidence of Sphaeropsis rot in storage. For the fruit inoculated 5 weeks before harvest, inoculations were done in mid-August in both seasons. In central Washington State, the weather in August is typically dry and very

Fig. 2. Effectiveness of thiabendazole (TBZ), fludioxonil (FLU) or pyrimethanil (PYR) as a postharvest treatment applied immediately after harvest in controlling Sphaeropsis rot (stem- and calyx-end rot) on stored 'Golden Delicious' apple in 2006–07 and 2007–08 seasons. Fruit were inoculated 2 weeks before harvest in the orchard with the conidial suspension of *Sphaeropsis pyriputrescens*, harvested, treated with postharvest fungicides, stored at 0°C, and monitored for decay development for up to 9 months after harvest. Columns within the same pattern group marked with the same letter are not significantly different at $P = 0.05$ according to Fisher's protected least significant difference.

hot, with average high temperature in August of 29°C in Wenatchee (21). A high level of *Sphaeropsis* rot incidence (58 to 75%) on these fruit 9 months after harvest indicated that latent infections resulting from the inoculations in mid-August can survive the hot summer in central Washington State, leading to *Sphaeropsis* rot in storage.

Because *S. pyriputrescens* can infect apple fruit during the fruit-growing season and remains latent in the fruit, part of our objectives was to determine whether a postharvest fungicide treatment is effective in controlling *Sphaeropsis* rot resulting from infections during the season. Our results suggest that, regardless of timing of infection, a postharvest treatment with one of the three fungicides applied immediately after harvest, such as a prestorage fungicide drench commonly used in commercial operations, can effectively control *Sphaeropsis* rot in stored apple fruit. However, it also is worthwhile to mention that the results in the present study pertain to fungicide treatments applied immediately after harvest. Fungicide treatments might not be equally effective as we documented here if the timing of postharvest fungicide application is delayed until a prepacking treatment after a period of time after harvest.

In summary, in this study, we documented that *S. pyriputrescens* isolates obtained from various sources were sensitive to all three registered apple postharvest fungicides, thiabendazole, fludioxonil, and pyrimethanil, and that a postharvest fungicide treatment with one of the three postharvest fungicides applied immediately after harvest was highly effective for controlling *Sphaeropsis* rot in stored fruit. Because *S. pyriputrescens* has been listed as a quarantine pathogen by some countries, a postharvest drench treatment with thiabendazole, fludioxonil, or pyrimethanil can serve as an effective mitigation measure for *Sphaeropsis* rot on apple fruit that are destined for export to those markets.

Acknowledgments

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