FINAL REPORT **YEAR 3/3**
WTFRC Project # PR-04-435 **WSU Project # 13C-3661-8367**

Project title: Phacidiopycnis Rot of Pears

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Objectives in 2002:
1. Conduct packinghouse surveys to determine the occurrence of Phacidiopycnis rot and rots caused by other pathogens in storage.
2. Test *in vitro* sensitivity of the fungus, *Phacidiopycnis piri,* to various fungicides in order to develop a fungicide program for control of this disease.
3. Evaluate the effectiveness and timing of postharvest treatments with fungicides (thiabendazole, TBZ and fludioxonil, Scholar) and biocontrol agents for control of Phacidiopycnis fruit rot.

Objectives in 2003:
1. Determine when *Phacidiopycnis piri* inoculum is available for fruit infection in the orchard.
2. Determine when fruits are infected in the orchard by the fungus *P. piri.*
3. Evaluate effects of selected fungicides on the fungus *P. piri.*
4. Evaluate effectiveness of fungicides and biocontrol agents to control Phacidiopycnis rot.

Objectives in 2004:
1. Determine when *Phacidiopycnis piri* inoculum is available for fruit infection in the orchard.
2. Determine seasonal susceptibility of pear fruit to infection by *P. piri* in the orchard.
3. Determine non-target effects of preharvest fungicides on *P. piri* in the orchard.
4. Evaluate effectiveness of pre- and postharvest treatments with fungicides in controlling Phacidiopycnis rot.

Significant findings during the past three years:
- A 2-year survey indicated that gray mold, Phacidiopycnis rot and blue mold are the three major postharvest diseases in d'Anjou pears grown in central Washington. In addition to gray mold and blue mold, Phacidiopycnis rot should be included as one of the targets for control of postharvest diseases in d'Anjou pears in the region.
- In addition to d'Anjou pears, Phacidiopycnis rot has also been found on Bosc and Comice pears.
- Phacidiopycnis rot causes three types of symptoms on pears: stem-end rot, calyx-end rot and wound-associated rot originating from infection of stem, calyx and skin wound of the fruit, respectively. Latent infection of stem and calyx of the fruit by the Phacidiopycnis fungus occurs in the orchard and symptoms develop during storage.
- *P. piri* was found to be associated with a canker and twig dieback disease of pear trees and widespread in pear-producing areas in the Pacific Northwest.
- The fruiting bodies (pycnidia) of *P. piri* containing viable conidia were available throughout the pear growing season, indicating that inoculum is likely not a limiting factor for fruit infection and rainfall or irrigation is more important to fruit infection since conidia of the fungus are water dispersed.
- None of the six fungicides (Flint, Ziram, Vangard, Procure and Dithane) were able to eliminate *P. piri* in twigs that were inoculated with the fungus two weeks before applications of fungicides, and

pycnidia containing viable conidia were produced on majority of fungicide-treated twigs, indicating that once twigs infected by *P. piri*, fungicides are generally not effective to control the fungus in diseased twigs.

- It appeared that d'Anjou pear fruit near harvest were more susceptible to infection by *P. piri*, but infection can also occur during early growing season when environmental conditions were met.
- Sensitivity of *P. piri* to various classes of fungicides was tested in vitro. Captan, Dithane, Procure and Ziram at the label and $1/10^{th}$ label rates, and TBZ and Scholar at the label, $1/10^{th}$ and $1/100^{th}$ label rates were effective in inhibiting mycelial growth of *P. piri*. Flint was not effective in inhibiting mycelial growth. Captan, Dithane, Flint, Scholar, TBZ, Vangard and Ziram at the label and $1/10^{th}$ label rates were effective in inhibiting conidial germination of *P. piri*. Procure was effective at the label rate but less effective at lower than $1/10^{th}$ the label rate. Information on sensitivity to various pre- and postharvest fungicides helps us develop fungicide programs for control of Phacidiopycnis rot.
- Ziram applied at 2 weeks before harvest significantly reduced both incidence and severity (size of the decay) of Phacidiopycnis rot that originated from infection of wounds on the fruit surface, but did not provide a satisfactory control.
- TBZ, Scholar and BioSave were very effective to control Phacidiopycnis rot originating from infection of wounds by *P. piri*. *Cryptococcus laurentii* strain 87-108 reduced Phacidiopycnis rot by 40-50% compared with the non-treated control. Aspire was not effective in controlling Phacidiopycnis rot in this experiment.
- Fruit dipped in TBZ one day after inoculation had either no or a very low percentage of Phacidiopycnis stem-end and calyx-end rot after six months of storage, indicating that TBZ drench after harvest may be effective in reducing the infection in stem and calyx that established near harvest.
- Inoculated fruit developed more Phacidiopycnis stem-end and calyx-end rot as the time of TBZ application was delayed. This indicates that TBZ applied on the packing line is likely not effective to eliminate established infection in pear stem and calyx of non-drenched fruit (fruit not treated with TBZ shortly after harvest) that had been stored for a period of time before packing.

Methods:

In 2001 and 2002, decayed fruit were collected during repacking and repackaging operations during March to May. Approximately 60 decayed fruit from each grower lot were randomly sampled. Decayed fruit were categorized by casual agents through visually examining symptoms, presence of sporulation of the pathogen or isolating from the diseased tissue.

Inoculum availability of *P. piri* was monitored in two commercial orchards from early spring to harvest during the pear growing seasons in 2002 and 2003 and in one orchard in 2004. Samples of dying or dead bark and dead fruit spurs were sampled and examined for the presence of fruiting bodies (pycnidia or apothecia) of the fungus.

To determine susceptibility of pear fruit to infection by *P. piri* during the pear growing season, pear flowers during bloom and fruit at different growth stages were inoculated with the fungus in 2003 and 2004. Fruit were harvested and stored in air for decay evaluation.

To evaluate the efficacy of postharvest treatments with fungicides and biocontrol agents for control of Phacidiopycnis rot, surface-disinfested pear fruit were wounded and inoculated. Three biocontrol agents, the *Cryptococcus laurentii* strain 87-108, BioSave, and Aspire, and two fungicides, thiabendazole (Mertect) and fludioxonil (Scholar), were tested. After inoculation, fruit were tray-packed and stored at 32°F in air.

To evaluate effectiveness of preharvest fungicides in controlling Phacidiopycnis rot originating from stem and calyx infections, fruit were inoculated with spore suspensions of the fungus during the pear growing season. Part of the inoculated fruit was sprayed with Ziram (at 14 days before harvest),

and the rest was not sprayed with Ziram. All fruit were harvested and stored at 32˚F in air for decay evaluation.

Experiments were conducted to evaluate whether postharvest treatments with TBZ can eliminate or reduce Phacidiopycnis rot originating from infections by the fungus of the stem and calyx of the fruit. The stem and calyx ends of the fruit were inoculated with spore suspensions of the pathogen. Fruit were kept in moist containers at room temperature overnight. Fruit were then stored in RA. Part of the inoculated fruit was treated with TBZ at 1, 10, 20, and 30 days after inoculation. Fruit were evaluated periodically for decay development.

In 2003 and 2004, experiments were conducted in two orchards to evaluate the effectiveness of five fungicides belonging to different classes in inhibiting production of pycnidia (fruiting bodies) of the fungus and the effects on survival of the fungus in diseased twigs.

Results and discussion:

Prevalence and incidence of Phacidiopycnis rot

A 2-year survey conducted in 2001 and 2002 indicated that gray mold caused by *Botrytis cinerea*, Phacidiopycnis rot caused by *Potebniamyces piri* (anamorph *Phacidiopycnis piri*) and blue mold account for an average of 60, 18 and 12% of the decay in the field bins (not drenched) (Table 1), respectively, and 25, 30 and 26% of the total decay on packed fruit in cardboard boxes, respectively (Table 2). The results indicate that gray mold, Phacidiopycnis rot and blue mold are the three major postharvest diseases on d'Anjou pears grown in Washington State, and decay control should target these three diseases in the region.

Table 1. Occurrence of postharvest diseases in the field bins sampled from mid-November 2001 to early January 2002.

Sampling period [1]	Phacidiopycnis rot	Gray mold	Blue mold	Mucor rot	Bull's eye rot[2]	Sprinkler rot[3]	Alternaria rot	Sphaeropsis rot
November	11.7	51.9	20.4	1.0	0	0.2	4.5	0
December	24.9	63.3	4.9	0.1	0	2.4	0.4	1.9
January	18.5	63.9	10.7	0	1.0	0.8	1.0	2.3
Mean	18.4	59.7	12.0	0.4	0.3	1.1	1.7	1.4

[1] Pears were stored in the field bins before packing. Samples were collected when packing was in operation. Ten, 13, and 10 samples of approximately 60 decayed fruit each were collected in November, December 2001, and January 2002, respectively. Each sample represents the fruit from one unique orchard.
[2] Bull's eye rot is caused by *Neofabraea* spp.
[3] Sprinkler rot is caused by *Phytophthora cactorum.*

Table 2. Occurrence of Postharvest diseases on packed fruit in the cardboard boxes sampled from March to May in 2001 and 2002[1]

Year	Production system	Phacidiopycnis rot	Gray mold	Blue mold	Mucor rot	Bull's eye rot[2]	Sphaeropsis rot	Others[3]
2001	Conventional	34.1	10.3	33.6	19.3	0.8	ND[4]	1.8
	Organic	22.8	35.7	23.5	1.4	7.4	ND	9.2
2002	Conventional	19.6	26.8	37.4	8.1	2.2	2.0	3.8
	Organic	42.2	25.7	8.2	0.5	7.4	4.5	11.5

[1] Pears were stored in cardboard boxes after packing. Samples were collected when repacking or repackaging was in operation. In 2001, 26 samples (6 organic and 20 conventional) of approximately 60 decayed fruit each were collected. In 2002, 39 samples (13 organic and 26 conventional) of approximately 60 decayed fruit each were collected. Each sample represents the fruit from one unique orchard.
[2] Bull's eye rot is caused by *Neofabraea* spp.

[3] Include Alternaria rot, Aureobasidium rot, Cladosporium rot, and other minor fruit rots caused by unidentified fungi and yeasts.
[4] ND=not determined.

Inoculum availability of *Phacidiopycnis piri* in the orchard.

Inoculum availability was monitored in two commercial orchards in 2003 and one in 2004. Results from the two years were similar. At each sampling time during the pear growing season in 2004, all sampled trees had viable pycnidia present on either bark or fruit spurs and 60-100% trees had pycnidia of the fungus containing fresh conidia inside (Table 3). From April to September, 65-72% of the bark samples and 16-31% of the spur samples had viable pycnidia; 19-54% of sampled bark and 8-22% of sampled spurs had pycnidia containing fresh spores. The data indicate that viable inoculum of *P. piri* appeared to be available during the pear growing season from April to September. This may also indicate that inoculum is likely not a limiting factor for fruit infection and that rainfall or irrigation is more important to fruit infection since spores of *P. piri* are water dispersed.

Table 3. Inoculum availability of *Phacidiopycnis piri* in a commercial Anjou pear orchards in 2004.

Date	Type of samples[a]	% Trees with pycnidia	% Trees with viable pycnidia	% Trees with fresh spores in pycnidia	% Samples with pycnidia	% Samples with viable pycnidia	% Samples with fresh spores in pycnidia	% Samples with viable apothecia
Apr-04	Spurs	90	80	70	18	17	14	0
	Bark	100	100	100	67	66	54	7
Jun-04	Spurs	100	100	70	34	31	17	1
	Bark	100	100	100	73	69	19	11
Jul-04	Spurs	100	100	90	34	29	22	1
	Bark	100	100	90	79	72	38	13
Sep-04	Spurs	90	70	60	24	16	8	2
	Bark	100	100	100	70	65	32	8

[a] At each sampling time, 10 trees were arbitrarily selected, and 10 samples each of dying or dead bark and dead fruit spurs were collected from each of 10 selected trees. The trees were at least two rows apart between rows and five trees apart within a row.

Seasonal susceptibility of Anjou pear fruit to infection by *P. piri* in the orchard.

In 2003, inoculation of fruit was conducted five times during the growing season. All inoculated fruit and fruit from the non-inoculated controls were harvested and stored in air at 32°F. The results indicate that d'Anjou fruit near harvest were more susceptible to infection at stem and calyx by *P. piri* (Fig. 1). The results also indicate that because *P. piri* causes latent infection and decay develops slowly during storage, Phacidiopycnis rot likely occurs more commonly on fruit after an extended period of storage. The experiment was repeated in 2004. At the time of submitting this report, fruit is still in storage for decay development. Results will be forthcoming later when experiments have been terminated.

Sensitivity of *Phacidiopycnis piri* isolates to selected fungicides.

Fungicide sensitivity data of representative isolates from the Wenatchee River Valley, the Hood River area, and the Medford area have been presented in previous reports. Sensitivity of mycelial growth and conidial germination has been tested. Here is a summary of the results. Captan, Dithane, Procure and Ziram at the label and $1/10^{th}$ label rates, and TBZ and Scholar at the label, $1/10^{th}$ and $1/100^{th}$ label rates were effective in inhibiting mycelial growth of *P. piri*. Flint was not effective in inhibiting mycelial growth. Captan, Dithane, Flint, Scholar, TBZ, Vangard and Ziram at the label and $1/10^{th}$ label rates were effective in inhibiting conidial germination of *P. piri*. Procure was effective at the label rate but less effective at lower than $1/10^{th}$ the label rate.

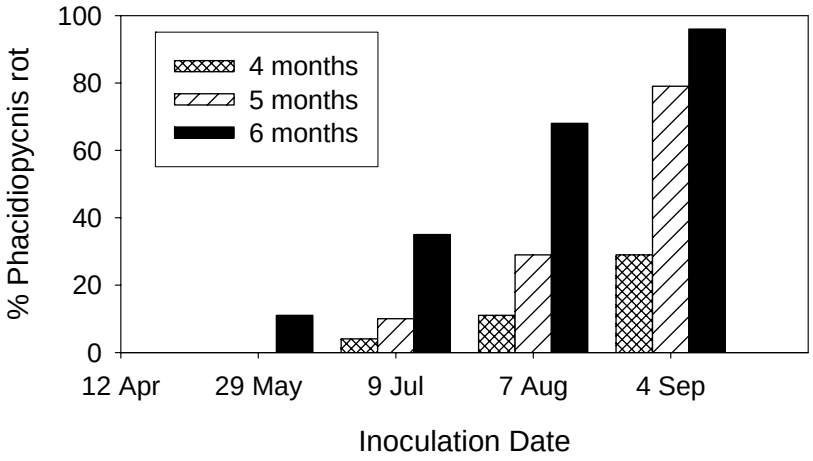

Fig.1. Susceptibility of d'Anjou fruit at different growth stages to Phacidiopycnis rot. Fruit were inoculated 5 times during the 2003 season and decay development was evaluated in 2004 (4, 5 and 6 months after the 2003 harvest).

Control of Phacidiopycnis rot originating from wound infections by *P. piri*.

Preharvest applications of fungicides to control Phacidiopycnis rot originating from infection of wounds on the fruit surface.

The trial was conducted in 2003. The data were presented in a previous report. Findings are summarized as follows. Ziram applied at 2 weeks before harvest significantly reduced both incidence and severity (size of the decay) of Phacidiopycnis rot that originated from infection of wounds on the fruit surface, but did not provide a satisfactory control (80% inoculated fruit developed Phacidiopycnis rot). Elevate and Procure applied at 2 weeks before harvest did not provide a satisfactory control in comparison with the non-treated treatment. Future research is needed to screen more effective fungicides and test effects of timing of applications on efficacy. Phacidiopycnis rot has the ability to spread from decayed fruit to the surrounding sound fruit in storage. Reducing decay incidence and severity (size of the decay) of Phacidiopycnis rot by preharvest fungicides would be beneficial in reducing secondary infections during the long-term storage of pears in the field bins.

Postharvest applications of fungicides and biocontrol agents to control Phacidiopycnis rot originating from infection of wounds on the fruit surface.

Experiments were conducted in 2002-03 and 2003-04. The first decay evaluation was done at two months after fruit inoculation. Scholar, TBZ and Biosave were effective in controlling Phacidiopycnis rot originating from infection of wounds of surface of the fruit (Fig. 2). No decay developed on fruit treated with TBZ and Scholar. *Cryptococcus laurentii* strain 87-108 reduced Phacidiopycnis rot by about 40-50% compared with the non-treated control. Aspire was not effective in controlling Phacidiopycnis rot in this experiment. The results indicate that a postharvest treatment of TBZ, Scholar or BioSave applied shortly after harvest would be effective in controlling Phacidiopycnis rot originating from wound infections by the fungus *P. piri*.

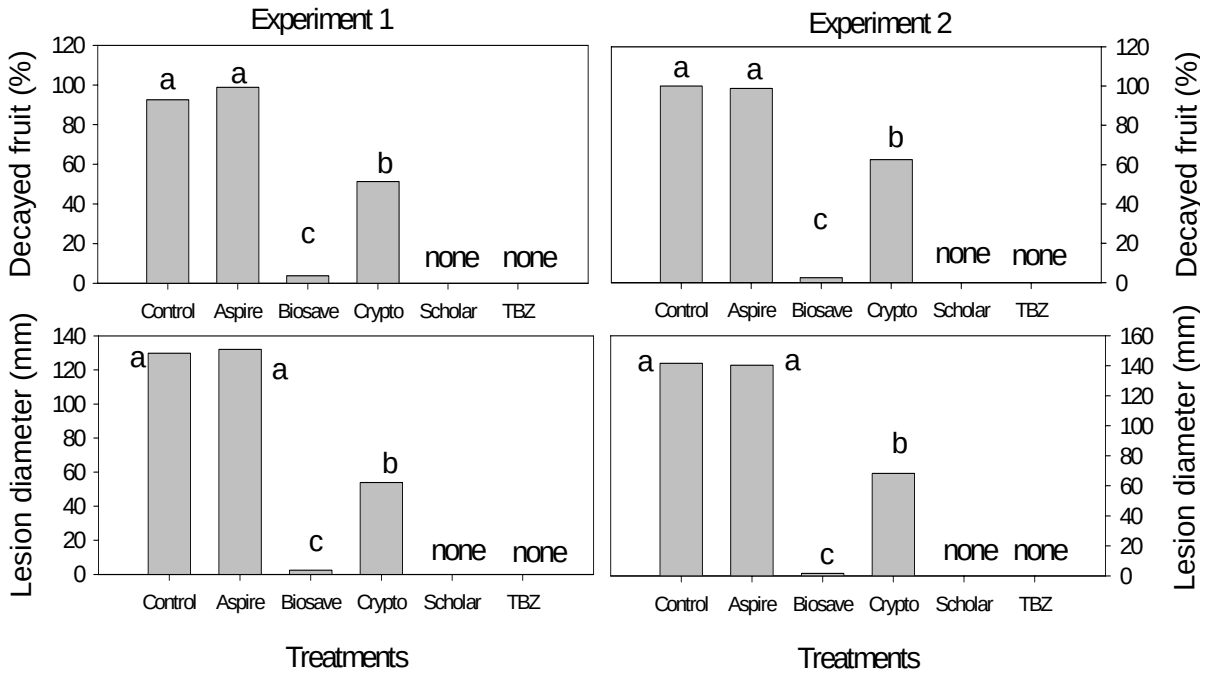

Fig. 2. Effectiveness of postharvest treatments with TBZ and three biocontrol agents, the *Cryptococcus laurentii* strain 87-108 (Crypto), BioSave and Aspire in controlling Phacidiopycnis rot originating from wound infections by *Phacidiopycnis piri* in 2003-04. Data of decay incidence after 3 months of storage are presented.

Effects of preharvest Ziram on Phacidiopycnis rot originating from infections of stem and calyx of the fruit.

The experiment was conducted in a research block. Fruit were inoculated with conidial suspensions of *P. piri* at three weeks before harvest, and Ziram was applied at two weeks before harvest. All fruit were harvested and stored in RA. Phacidiopycnis rot symptoms (stem-end rot or calyx-end rot) developed after three months of storage. By six and a half months after harvest, 66% of inoculated fruit that were not treated with fungicides before harvest developed Phacidiopycnis rot originating from infection at either stem or calyx of the fruit (Table 4). Ziram applied at 14 days before harvest reduced Phacidiopycnis rot by 32% compared with the non-fungicide treated control.

The experiment conducted in 2004 is still in progress for decay evaluation and results will be forthcoming after the experiment is completed.

Table 4. Effects of a preharvest application of ziram on Phacidiopycnis rot originating from infection of stem and calyx of the fruit.

Treatment	% of the fruit that developed Phacidiopycnis rot			
	12 Jan 2004 (4 months in storage)	11 Feb 2004 (5 months in storage)	11 Mar 2004 (6 months in storage)	26 Mar 2004 (6.5 months in storage)
No fungicides	3	25	54	66
Ziram at 14 days before harvest	3	16	36	45

Effects of timing of postharvest TBZ on Phacidiopycnis rot originating from infections of stem and calyx of the fruit.

Experiments were conducted in the 2002-03 and 2003-04 storage seasons. Results from the 2-year experiments were very similar. The results from one experiment conducted in 2003-04 are presented in Fig. 3. Inoculated fruit treated with TBZ at 1, 10 and 20 days after inoculation had significantly lower percentages of Phacidiopycnis stem-end rot compared with those that were either not treated with postharvest fungicides or treated with TBZ 30 days after inoculation. Significantly lower percentages of fruit treated with TBZ within 20 days after inoculation developed Phacidiopycnis calyx-end rot compared with the non-treated control. But TBZ applied at 30 days after inoculation did not significantly reduce Phacidiopycnis calyx-end rot compared with the nontreated control. The results from this study suggest that a postharvest drench with Mertect (TBZ) may be effective to reduce newly established infection in stem and calyx of the fruit. However, TBZ applied on the packing line is likely not effective to eradicate established infection in pear stem and calyx of non-drenched fruit that had been stored for a period of time before packing.

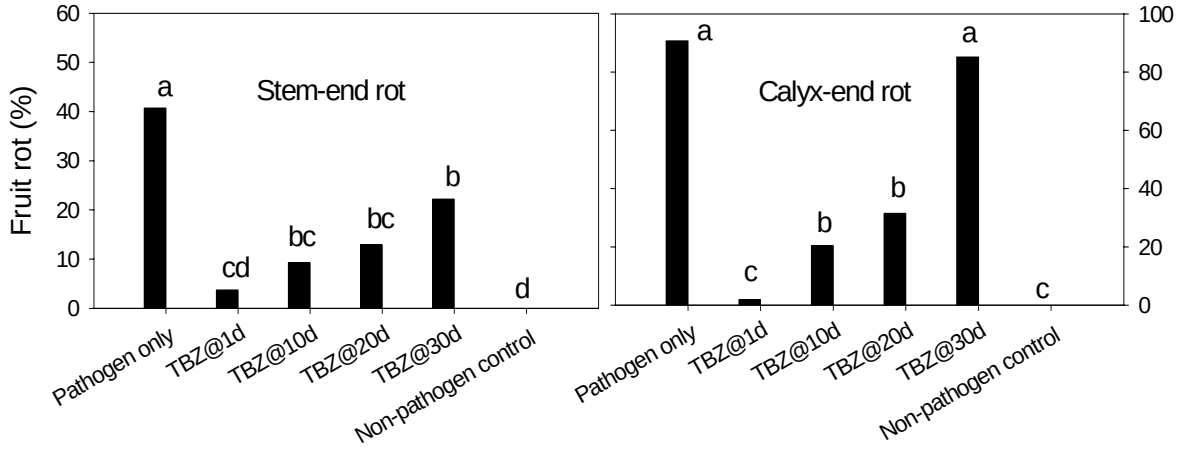

Fig. 3. Effects of timing of a postharvest treatment with thiabendazole on Phacidiopycnis stem-end and calyx-end rot in 2003-04. Inoculated fruit were treated with TBZ at 1, 10, 20, and 30 days after inoculation. Data represent the decay after five months of storage. Bars with different above them are significantly different.

Evaluation of Penbotec for control of Phacidiopycnis rot originating from wound infections.
In 2004, an experiment was conducted to evaluate Penbotec for control of Phacidiopycnis rot resulting from infection of wounds on surface of the fruit. The purpose was to determine whether a postharvest drench with Penbotec is effective to control wound infections. The experiment is still in progress at the time of submitting this research report. Results from this experiment will be forthcoming.

Effects of fungicides on production of fruiting bodies and survival of *P. piri* in diseased twigs.
In both 2003 and 2004, five fungicides belonging to different classes were evaluated for the effectiveness in inhibiting production of pycnidia (fruiting bodies) of the fungus and the effects on survival of the fungus in diseased twigs. The purpose of this experiment was to determine if *P. piri* has established in twigs or bark tissues, whether fungicide treatments in the orchard can control the fungus in diseased twigs and inhibit production of fruiting bodies (pycnidia) of the fungus in diseased twigs. We found that none of these five fungicides were able to eliminate the fungus in diseased twigs and majority of fungicide-treated twigs were able to produce pycnidia containing viable conidia

(Table 5). The results suggest that once twigs infected by *P. piri*, fungicides are generally not effective to control the fungus in diseased twigs.

Table 5. Effects of fungicides on the production of pycnidia and survival of *P. piri* in diseased twigs in 2004.

Orchard	Treatment[1]	% Twigs with viable pycnidia	% Twigs from which *P. piri* was recovered
ST 7	Nontreated	81.3	100
	Flint	87.5	100
	Ziram	93.8	100
	Vangard	81.3	100
	Procure	92.9	100
	Dithane	93.3	100
TFREC 8	Nontreated	87.5	100
	Flint	81.3	100
	Ziram	100.0	100
	Vangard	85.7	100
	Procure	81.3	100
	Dithane	87.5	100

[1] All twigs were freeze-treated and inoculated with mycelial plugs of *P. piri* on April 22. Twigs in the fungicide treatments were applied with fungicides at 2 and 4 weeks after inoculation. All twigs were removed from trees at 6 weeks after inoculation and subjected to examination for the presence of pycnidia and isolation of the fungus from diseased tissues.

Acknowledgments

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Budget:

Project title: Phacidiopycnis Rot of Pears
PI: Chang-Lin Xiao
Proposed project duration: 2002-2004
Current year: 2004
Project total (3 years): $62,281
Current year request: $25,081

Current year breakdown:

Item	2002	2003	2004
Salaries [1]		14,000	14,560
Benefits (18%)		2,520	2,621
Wages [2]	8,000	2,500	2,500
Benefits (16%)	1,280	400	400
Supplies [3]	2,500	4,000	4,000
Travel [3]	1,000	1,000	1,000
Total	12,780	24,420	25,081

[1] Salary for a 0.49 FTE technical helper (Associate in Research).
[2] Wages for time-slip in summer and during harvest.
[3] Culture 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.
[4] We will be using a leased vehicle.