Biological Control of Phacidiopycnis Rot in ‘d’Anjou’ Pears

C.L. Xiao
US Department of Agriculture
Agricultural Research Service
Parlier, California 93648
USA

R.J. Boal
Washington State University
Tree Fruit Research and Extension Center
Wenatchee, Washington 98801
USA

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Abstract
Phacidiopycnis rot, caused by Phacidiopycnis piri, is a recently reported postharvest fruit rot disease of pears (Pyrus communis) in the US, and a major disease of ‘d’Anjou’ pears grown in Washington State. Phacidiopycnis rot can originate from infection of wounds on the fruit. In this study, two biocontrol agents, BioSave (a Pseudomonas syringae strain) and Cryptococcus laurentii strain 87-108, were compared with the conventional fungicide thiabendazole (TBZ) for their efficacy in controlling Phacidiopycnis rot. ‘D’Anjou’ pear fruit were surface disinfected, wounded with a finishing nail head, treated with one of the biocontrol agents or TBZ, and inoculated with conidial suspension of P. piri. An untreated control where fruit were inoculated with the pathogen was also included in the trial. The experiment was conducted twice using fruit from different orchards where no fungicides were used. Each treatment contained four 20-fruit replicates. Inoculated fruit were placed on fiber fruit trays wrapped with perforated bags and stored in cardboard boxes at 0°C for three months, at which time decay was assessed. Over 92% of the fruit in the untreated control developed Phacidiopycnis rot. BioSave and C. laurentii significantly reduced incidence of Phacidiopycnis rot by 96-98 and 38-45%, respectively, compared with the control. BioSave was more effective than C. laurentii. No decay was observed on TBZ treated fruit, but there was no statistical difference in decay incidence between BioSave and TBZ treatments. The results suggest that BioSave is effective in controlling Phacidiopycnis rot originating from wound infections by P. piri and can provide a level of control of Phacidiopycnis rot comparable with that of TBZ.

INTRODUCTION
In the US, ‘D’Anjou’ pears (Pyrus communis) are produced primarily in the Pacific Northwest. Fruit are either packed shortly after harvest or stored in field bins and packed when there are market demands. Postharvest fruit rot diseases can cause significant economic losses if they are left uncontrolled.

Phacidiopycnis rot, caused by Phacidiopycnis piri, is a recently reported postharvest fruit rot disease of pears in the US and a major disease of ‘d’Anjou’ pears grown in Washington State (Xiao and Boal, 2004). Phacidiopycnis rot can originate from infection of wounds on the fruit.

For conventional fruit, postharvest fungicides applied prior to storage and during packing are commonly used to control postharvest rots (Xiao and Kim, 2010). For organic fruit, few options for postharvest disease control are available. Biological control is a promising approach for control of postharvest diseases in fresh fruits, and some biocontrol agents such as BioSave, have been registered for postharvest use on fresh fruits (Janisiewicz and Korsten, 2002). Various experimental biocontrol agents have been shown to be effective against major postharvest pathogens (Arras and Maltoni, 2004). Cryptococcus laurentii strain 87-108 was originally isolated from apple fruit and is effective for control of gray mold in apples (Roberts, 1990). The objective of this study was to evaluate biocontrol agents BioSave and Cryptococcus laurentii strain 87-108 for their effectiveness in controlling Phacidiopycnis rot in ‘d’Anjou’ pears.

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MATERIALS AND METHODS

‘D’Anjou’ pear fruit were harvested at commercial maturity from one commercial pear orchard (for experiment 1) located in Leavenworth, Washington and one research orchard (for experiment 2) located in Orondo, Washington. No fungicides were applied to the fruit in these orchards. After harvest, fruit were stored at 0°C in air for about a month until use in the experiments. Prior to the experiments, fruit were surface disinfected in 0.6% sodium hypochlorite solution for 5 min, rinsed three times with fresh water, and air dried.

Isolate CLX210 of *P. piri* obtained from a Phacidiopycnis rot-decayed pear fruit was used in this study. Inoculum of *P. piri* used for fruit inoculation was prepared following the protocol of Xiao and Sitton (2004). Pycnidia produced on oatmeal agar cultures grown under 12-h light/12-h dark for 10-12 weeks were removed from the medium surface with a scalpel and ground with a pestle and mortar to release conidia. Sterile water was added to the mortar to make a conidial suspension and conidia were filtered through four layers of cheesecloth. The concentration of the conidial suspension was adjusted to $1 \times 10^6$ conidia ml$^{-1}$ with a hemacytometer. Tween 20 was added to a final concentration of 0.001%.

*Cryptococcus laurentii* strain 87-108 was cultured on yeast malt dextrose agar for 2 days at 20°C, after which yeast colonies were scraped from the medium and suspended in sterile distilled water. The yeast suspension was adjusted to 2% transmittance at 595 nm with a spectrophotometer resulting in concentrations of $6.8 \times 10^5$ and $8.1 \times 10^5$ cells ml$^{-1}$ for experiment 1 and experiment 2, respectively. BioSave 110 solution (JET Harvest Solutions, Longwood, Florida, USA) was prepared according to the manufacturer’s directions, and the concentration of the resulting solution was 1.7 to $2.1 \times 10^6$ cells ml$^{-1}$. A formulated thiabendazole (TBZ) (Mertect 340F; Syngenta Crop Protection, Greensboro, North Carolina, USA) was used at label rate of 1.25 ml L$^{-1}$.

An artificial wound was made on each fruit with a sterile 5 mm diameter finishing nail head to a depth of 4 mm. The fruit were treated with either BioSave 110, *Cryptococcus laurentii* strain 87-108, or TBZ by placing 30 µl of the appropriate solution on each wound. Fruit treated with sterile water was used as a control. Approximately 30 min after application of the biocontrol agents, TBZ, or water, each wound was inoculated with 30 µl of conidial suspension of the pathogen as described above. There were four 20-fruit replicates for each treatment. All inoculated fruit were incubated at 0°C in air. Lesion diameter (decayed area) on each fruit was recorded 12 weeks after inoculation and incidence of decay was determined. The experiment was conducted twice.

Analysis of variance was performed with SAS PROC GLM (Version 9.2, SAS Institute, Cary, North Carolina, USA) to determine whether incidence and severity (lesion size on the fruit) of Phacidiopycnis rot were significantly different among the postharvest treatments. Means were separated by Waller-Duncan K-ratio $t$ test with K-ratio = 100 ($P=0.05$). All incidence (percentage) data were arcsine-transformed prior to analysis.

RESULTS AND DISCUSSION

After storage at 0°C for 12 weeks, 92.5 and 100% of the fruit in the non-treated control developed Phacidiopyc尼斯 rot in experiments 1 and 2, respectively (Fig. 1). In comparison, less than 4% of the fruit treated with BioSave developed Phacidiopyc尼斯 rot symptoms. BioSave and *C. laurentii* significantly reduced incidence of Phacidiopyc尼斯 rot by 96-98 and 38-45%, respectively compared with the non-treated control (Figs. 1 and 2). BioSave was more effective than *C. laurentii*. No decay was observed on TBZ treated fruit, and there was no statistical difference in decay incidence between BioSave and TBZ treatments. Similar differences among the treatments also were observed for decay severity (lesion size among the decayed fruit) (Figs. 1 and 2).

The results suggest that BioSave is effective in controlling Phacidiopyc尼斯 rot originating from wound infections by *P. piri* and can provide a level of control comparable with that of TBZ.
**Literature Cited**


**Figures**

Fig. 1. Effectiveness of BioSave (a.i. *Pseudomonas syringae* strain), *Cryptococcus laurentii* strain 87-108 (Crypto) and thiamendazole (TBZ) in controlling Phacidiopycnis rot (disease incidence) on ‘d’Anjou’ pears. Bars with different letters above them for each experiment are significantly different \((P=0.05)\).
Fig. 2. Effectiveness of BioSave (a.i. *Pseudomonas syringae* strain), *Cryptococcus laurentii* strain 87-108 (Crypto) and thiabendazole (TBZ) in controlling Phacidiopycnis rot (disease severity) on ‘d’Anjou’ pears. Lesion size was the average of decayed fruit only. Bars with different letters above them for each experiment are significantly different ($P=0.05$).