Use of Filtration for Removal of Conidia of *Penicillium expansum* from Water in Pome Fruit Packinghouses

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ABSTRACT

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Removal of conidia of *Penicillium expansum* from aqueous suspensions with sieves of 2.0 mm to 10 μm and with a triple-stage cartridge filter unit with 5-, 1-, and 0.45-μm terminal filters was studied. The effectiveness of sand filters and cartridge filters for removal of conidia also was evaluated in commercial apple drenchers and packinghouse flumes. The sieves were effective for removal of conidia only when 5–20 mg/ml of soil was added to the suspension, indicating that conidia frequently were bound onto soil particles. In the packinghouse, sand filters did not reduce the concentration of *P. expansum* in the flume water. A triple-stage filter unit with a 0.45-μm terminal cartridge filter removed 99% of *P. expansum* conidia from aqueous suspensions in the laboratory, and a similar four-element cartridge unit removed 92% of *P. expansum* conidia from the carrier water suspension used in commercial drenchers. The filter systems were advantageous for prolonging the use of drencher suspensions and water in packinghouses but currently lack the capacity to provide continuous, unaided reduction of concentrations of conidia to levels that would control blue mold.

Additional keywords: decay, postharvest

Blue mold, caused by *Penicillium expansum* Link, is a major decay of pome fruits (3). Conidia of *P. expansum* are found in soil, on plant surfaces, and in air (5) and are transferred to dump tank and flume water in packinghouses on contaminated wooden picking bins and fruit (12). Populations of *P. expansum* in water in pome fruit packinghouses vary considerably and are affected by the amount of fruit and bin contamination, the volume of fruit processed, and the use of fungicides and disinfestants. Levels of conidia of *P. expansum* of 200–12,000/ml (6) and of *Penicillium* spp. of 300–28,000/ml (2) have been reported in apple cleaning solution and packinghouse water, respectively. In the Pacific Northwest, 44% of samples of dump tank water from apple and pear packinghouses contained between 10 and 100 conidia of *P. expansum* per milliliter (1). In another study in Oregon and Washington, populations of *P. expansum* in the dump water varied throughout the packing season from 56 to 4,167 conidia per milliliter (10). Quantitative relationships have been established between concentrations of *P. expansum* conidia in water and decay of pear fruits (9).

The two most common disinfestants used to reduce spore levels in dump tank and flume water are chlorine (sodium hypochlorite) and sodium-*o*-phenylphenate (SOPP) (1,14). Chlorine is inexpensive but is corrosive to equipment and will not kill spores lodged in injured tissues (1,13). SOPP disinfests injured tissues to a limited extent but occasionally injures fruit if not thoroughly rinsed (1). SOPP currently is undergoing reregistration. Other methods to reduce spore levels include ozone (11) and chlorine dioxide (13), but these methods also have limitations and are not widely used.

Physical removal of fungal decay spores from water has received little attention. In Australia, a combination of sand, cellulose cartridge, and ceramic filters has been used successfully to remove fungal spores from irrigation water (4). In Canada, dump tank water in an apple packinghouse was passed through two Jacuzzi sand filters, and levels of *P. expansum* were reduced 59–88% (8). However, the filter system was considered too erratic to provide reliable water sanitation.

In this research, we first studied removal of conidia of *P. expansum* from aqueous suspensions containing three amounts of soil using a series of sieves from 2.0 mm down to 0.053 mm with a 10-μm terminal Millipore filter. Second, a prototype commercial triple-stage cartridge filter with terminal filter sizes of 5, 1, and 0.45 μm was evaluated. Finally, a commercial version of the triple-stage filter and two sand filters were evaluated in packinghouses.

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MATERIALS AND METHODS

Removal of conidia with sieves. *P. expansum* isolate 46 was cultured on potato-dextrose agar acidified with 1.5 ml/L of lactic acid (APDA). Cultures 1–2 wk old were flooded with sterile distilled water (SDW), and suspensions were adjusted to 3,000 conidia per milliliter with the aid of a hemacytometer. Hood River sandy loam soil from the top 2.5 cm in a pear orchard was mixed for 90 min in a twinshell blender, passed through a 5-mm screen, and heated at 80 C for 10 min in a microwave oven with a temperature probe. Soil was added to the suspensions of conidia at 0, 5, 10, and 20 mg/ml. (Average solids concentration in dump water of several packinghouses was 4.97 mg/ml and ranged from 0.37 to 15.13 mg/ml [R. A. Spotts, *unpublished*].) After suspensions were thoroughly mixed, a 0.1-ml sample was removed and diluted in 9.9 ml of SDW, and 0.5 ml was plated on each of five replicate petri dishes containing APDA. The suspension was passed through a series of standard testing sieves with mesh openings of 2.0, 1.0, 0.50, 0.25, 0.125, and 0.053 mm, then through a 10-μm Millipore filter. As the suspension was passed sequentially through the filters, a sample was removed after each filter, diluted, and plated as described above. Petri dishes were incubated at 20 C, and colonies were counted after 48 hr. The experiment was conducted twice with each concentration of soil in the suspension. The relationships between cumulative percent removal of conidia or soil and filter pore size were analyzed with linear regression. In addition, the percentage of conidia removed by each filter was calculated, and percentage values were transformed to arcsine square root before analysis of variance with sieve size and soil level as fixed effects and trial as a random effect. The pooled trial × treatment sums of squares were used to calculate the error mean square terms. Separation of treatment means was done with Tukey's HSD test (15).

Removal of conidia with a triple-stage cartridge filter unit. A suspension containing 1.0×10^4 conidia of *P. expansum* per milliliter was prepared in 113.5 L of tap water. This suspension was circulated through a 45-μm nylon mesh bag, through a 20-μm polypropylene cartridge filter (Hytrex GX series, Osmonics Inc., Minnetonka, MN), then through a terminal cartridge filter of 5.0 (Hytrex), 1.0 (Hytrex), or 0.45 μm (Microfiberglass, Keystone Filter Division, Hatfield, PA) and back into the main tank. In tests with the 1.0- and 0.45-μm terminal filters, conidia were suspended in either water or an emulsion of diphenylamine (No Scald DPA, Atochem North America, Inc., Monrovia, CA) and calcium chloride (Stopit, Phosyn International Ltd., Packington, York, UK) at 969 and 1,600 mg a.i./L, respectively. Controls included water and DPA plus calcium chloride without spores added to check the level of disinfestation of the system between trials. Water or emulsions were circulated through the three filters at 51 L/min, with the pressure at the terminal filter of 2.8 kg/cm^2. Prior to filtration and after 1 and 2 min of filtration, 0.2 ml of suspension exiting the terminal filter was removed and diluted in 9.8 ml of SDW, and 0.1 ml was plated on each of five replicate dishes of APDA. Dishes were incubated at 20 C and colonies counted after 72 hr. Each suspension-terminal filter combination experiment was conducted twice. Percent conidia removal was calculated, data were transformed to square root of (100 − percent removed) to obtain a random distribution of residuals (15), and data were analyzed with analysis of variance and Tukey's HSD test.

Packinghouse evaluations. Two commercial sand filters (Triton TR 140, Pac Fab, Inc., El Monte, CA) were evaluated in an apple packinghouse. Each filter contained 114 kg of pea gravel and 227 kg of No. 20 silica sand and had a filter area of 0.641 m^2. Flume water was pumped through the filter at 530 L/min. Water entering and exiting each filter was sampled weekly from 21 November 1986 to 11 May 1987 (20 samples). Each sample was plated on three replicate dishes containing APDA as described in the sieve experiments above. Dishes were incubated at 20 C, and colonies of *P. expansum* were counted after 5 days. Data were transformed to log (colony forming units per milliliter +1) and analyzed with a paired t test.

A commercial multistage filtering system connected to a drive-through bin drencher at several apple packinghouses was evaluated. The drenching liquid was a water suspension of DPA at 1,938 mg a.i./L and thiabendazole (Mertect 340 F) at 528 mg a.i./L. The first stage of filtration consisted of a 0.51-mm screen and liquid cyclone units (Maxicleaner, Rush Consultants, Wenatchee, WA) operated at 757 L/min, continually recycling the liquid from tanks containing 5,700–7,500 L to remove leaves, stems, and other large debris. Periodically, after 2,000–3,000 bins had been processed, the liquid in the drencher holding tank was emptied into another storage tank, and the suspension was pumped through a final set of filters (Zero discharge unit, Rush Consultants) at 37.9 L/min. These filters were the same as those used in the triple-stage cartridge experiment above and consisted of four elements in a step-down series: 100- and 45-μm nylon mesh filter bags, a pair of 5-μm polypropylene cartridge filters, and a terminal pair of either 1.0-μm polypropylene or 0.45-μm glass fiber cartridge filters. Samples were taken before and after filtration on five occasions where 1.0-μm terminal filters were in use and on three occasions with 0.45-μm filters. Samples were dilution plated and incubated, and colonies of *P. expansum* were enumerated as described above. Data were transformed to square root values and analyzed with an unpaired t test.

RESULTS

Removal of conidia with sieves. Addition of soil to the conidial suspension significantly (P = 0.01) increased the percent removal of conidia. However, no significant differences in the percent removal of conidia were observed among suspensions containing 5, 10, or 20 mg of soil per milliliter. Filtration through the 2.0-mm sieve removed 45.7% of the conidia from suspensions containing soil, and the concentration of conidia after filtration through the 2.0-mm sieve was significantly (P = 0.01) less than the concentration before filtration. This level of filtration of suspensions of conidia without soil produced only a 19.6% reduction, which was not significant. Additional filtration of conidial suspensions with or without soil through filters down to 0.053 mm did not result in any additional removal of conidia (Table 1), and regressions of conidial removal on pore size were not significant. The 10-μm filter appeared to increase removal of conidia, but the increase was not significant. When a suspension containing 20 mg of soil per milliliter was passed through all of the filters, 99.5% of the soil but only 75% of the conidia were removed. Regression of cumulative soil removal on pore size was significant (P = 0.01).

Removal of conidia with a triple-stage cartridge filter unit. No differences in removal of conidia were measured between samples taken 1 or 2 min after the start of filtration, and data were combined for statistical analysis. Percent removal of conidia ranged from 78.3% with a 5-μm terminal filter to 99.7% with the 0.45-μm filter (Table 2). There was no significant difference between filtration efficiency with water or the DPA-calcium emulsions.

Packinghouse evaluations. The average number of *P. expansum* colony-forming units per milliliter in water increased significantly (P = 0.05) from 547 before filtration through sand filter unit No. 1 to 725 after filtration. With sand filter unit No. 2, 458 cfu/ml were detected in water before and 441 cfu/ml after filtration (difference not significant, P = 0.05). There was no significant difference (P = 0.05) in the number of *P. expansum* colony-forming units detected in commercial drencher water before and after filtration through the four-element cartridge filter unit when the terminal filter was 1.0 μm (1,153 and 1,123 cfu/ml, respectively). However, significantly more (P = 0.03) conidia

Table 1. Filtration of conidial suspensions of *Penicillium expansum* with and without soil through sieves

Filter pore size	Cumulative percent conidia removed[x] No soil	Cumulative percent conidia removed[x] Soil[z]	Cumulative percent soil removed[y]
2.0 mm	19.6	45.7	15.5
1.0 mm	15.9	53.8	32.8
0.50 mm	19.9	56.0	53.7
0.25 mm	26.0	57.3	79.2
0.12 mm	26.9	54.0	91.2
0.053 mm	22.7	53.0	96.5
10.0 μm	39.4	73.4	99.5

[x]Regression of cumulative percent conidia removed on filter pore size not significant (P = 0.01).
[y]Values based on suspension containing 20 mg of soil per milliliter. Regression equation for cumulative percent soil removed (Y) on filter pore size (X): $Y = -43.5X + 91.35$. Regression significant at $P = 0.01$.
[z]Means of treatments containing 5, 10, and 20 mg of soil per milliliter.

Table 2. Filtration of conidia of *Penicillium expansum* from aqueous suspensions with a triple-stage cartridge system

Terminal filter size (μm)	Suspension[y]	Percent conidia removed[z]
5.0	Water	78.3 a
1.0	Water	92.2 b
1.0	DPA + calcium	88.0 b
0.45	Water	99.7 c
0.45	DPA + calcium	99.5 c

[y]DPA at 969 and calcium chloride at 1,600 mg a.i./L.
[z]Numbers followed by the same letter are not significantly different at $P = 0.05$ according to Tukey's HSD test.

were removed from drencher suspensions filtered through the four element units when the terminal filter was 0.45 μm (617 vs. 50 cfu/ml before and after filtration, respectively).

DISCUSSION

Sand filters were relatively ineffective for direct reduction of conidia of *P. expansum* from flume water. The sand filters that were used commercially were designed to remove particles larger than about 0.5 mm. In our laboratory sieve experiments, 56% of conidia and 53.7% of soil were removed from suspensions that were passed successively through the 2.0-, 1.0-, and 0.5-mm sieves. In the packinghouse, however, there was not a significant reduction in the number of colony-forming units detected in the one filter and there was an increase in the number detected in the other. Although sand filters are backwashed regularly, they still may build up organic matter such as pieces of fruit, leaves, and stems that may serve as a food base for growth and sporulation of fungi inside the filter unit. In addition, build up of the organic matter may cause nonuniform water flow or "channeling" through the filter, which results in decreased efficiency. Sholberg and Owen (8) reported that a sand filter system in an apple packinghouse did not always work efficiently and was not able to lower the propagule concentrations of *P. expansum* enough to remove the risk of infection. However, less chlorine was required in filtered water than in nonfiltered water to maintain the desired concentration of chlorine. Filtering also prolonged the use of dump tank water and lengthened packing runs before tank cleaning was necessary.

The cartridge filter system has potential for effective removal of decay spores and, thus, increased decay control. However, effectiveness is closely related to pore size of the terminal filter. In our laboratory tests, the filter system with a 1.0-μm terminal filter removed about 90% of *P. expansum* conidia but a similar unit used with a commercial drencher removed only 3% of conidia. The 1.0-μm filter is nominally rated and removes only 80–85% of 1.0-μm particles.

The cartridge filter system with the 0.45-μm terminal filter was very effective for removal of conidia in both laboratory and commercial tests, reducing the level of conidia by 92–99%. However, if this system is to become more useful for spore removal and decay control in packinghouse drenchers, dump tanks, and flumes, the capacity must be increased well beyond the current 38 L/min rate. The volume of water in drenchers and flumes may exceed 3,700 and 18,000 L, respectively. In addition, 1.0-μm terminal filters remove about 20% and 0.45-μm filters remove an even greater but unknown amount of thiabendazole, the most common fungicide used in drencher water for decay control. Consequently, fungicide concentration must be monitored and adjusted continually (H. Rush, *personal communication*).

As with the sand filters, the cartridge filter units effectively increase the time that drencher suspensions can be used. Because drencher water may contain DPA, calcium chloride, and thiabendazole, reduction of the amount of waste water that is generated and must be disposed of is a positive benefit of filtration. During harvest, bottoms of bins often are covered with soil and debris (7). Filter life, and possibly filtration rate, may be increased by improved bin sanitation.

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