Populations, Pathogenicity, and Benomyl Resistance of *Botrytis* spp., *Penicillium* spp., and *Mucor piriformis* in Packinghouses

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ABSTRACT

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Populations of *Botrytis* spp., *Penicillium* spp., and *Mucor piriformis* were determined during two seasons in air and dump-tank water of nine apple and pear packinghouses. Populations of all fungi varied considerably among packinghouses. Spores of *Penicillium* spp. were more abundant in air and dump water than spores of *Botrytis* spp. or *M. piriformis*. Spores of *Penicillium* spp. and *M. piriformis* in dump water increased as the packing season progressed, suggesting that decaying fruit stored in bins and processed later in the season increased propagule levels more than did debris brought into packinghouses from orchards early in the season. Selected isolates were characterized for pathogenicity and virulence on Anjou pear fruit and resistance to benomyl. Sixty, 72, and 89% of *Botrytis* spp., *Penicillium* spp., and *M. piriformis* isolates, respectively, were pathogenic on pear fruit. The percentage of pathogenic *Penicillium* spp. isolates resistant to benomyl also increased later in the season. Benomyl-resistant isolates of *Penicillium* spp. were less virulent than benomyl-sensitive isolates. The percentage of *Penicillium* spp. isolates resistant to benomyl in the Mid-Columbia region has not increased during the last 5 yr.

Botrytis cinerea Pers. ex Fr., *Mucor piriformis* Fischer, and *Penicillium expansum* Lk. ex Thom. cause serious postharvest losses to apples (*Malus sylvestris* Mill.) and pears (*Pyrus communis* L.) in the Pacific Northwest (5,13). These fungi have been found in soil, surface plant residues, air (10), and packinghouse dump-tank water (3,4,6,9). Pear fruit increases in susceptibility to decay during the last month before harvest (1,7,15), and inoculation can occur as fruit floats through contaminated dump-tank water. A positive relationship between decay and spore population in the water was reported for apple (6). Most packinghouses use chlorine or sodium ortho phenylphenate (SOPP) in dump water to reduce spore levels and control decay (4). In addition, benomyl is applied to fruit before cold storage to control decay caused by *B. cinerea* and *P. expansum* (3). However, benomyl-resistant strains of *B. cinerea* and *P. expansum* were found in Oregon and Washington in 1978 in dump water and decayed fruit (3).

This study reports changes in the populations of *Botrytis* spp., *Penicillium* spp., and *M. piriformis* during two packing seasons in nine apple and pear packinghouses in the Mid-Columbia region of Oregon and Washington. Pathogenicity of these fungi and virulence and benomyl resistance of *Botrytis* spp. and *Penicillium* spp. isolates also are reported.

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MATERIALS AND METHODS

Populations of *Botrytis* spp., *Penicillium* spp., and *M. piriformis* were determined in air and dump water in nine apple and pear packinghouses weekly from September 1981 to April 1982 and monthly from October 1982 to March 1983. Air populations were determined by removing the lid for 5 min from 10-cm-diameter petri plates containing 15 ml of potato-dextrose agar (Difco) acidified with 1.5 ml of 85% lactic acid per liter (APDA). Dump-water populations were determined by placing 0.5 ml of a 1:99 dilution of dump-tank sample with water on APDA. Three replicate plates were used for both air and water samples in each packinghouse. Plates were incubated at 20 C and colonies counted after 5 days. At each sampling time, chlorine (2) or SOPP concentration (Steri-Seal, Inc., Wenatchee, WA, *personal communication*) in the dump water was determined with sodium thiosulfate titration.

About six isolates of *Botrytis* spp., *Penicillium* spp., and *M. piriformis* from air and six from water from each packinghouse were selected on each sampling date for pathogenicity tests. To determine pathogenicity, Anjou pear fruits were disinfested with 95% ethanol and inoculated with each isolate by placing spores and hyphae into a 1-mm-deep wound. Decay was evaluated after 3–5 days at 20 C in a moist chamber. Virulence of *P. expansum* isolates was determined by wound-inoculating Anjou pear fruits with 2.5×10^6 conidia per milliliter and comparing lesion size with lesions produced by *P. expansum* isolate 46 recovered from an Anjou pear fruit in 1980 at the Mid-Columbia Experiment Station and characterized as sensitive to benomyl. To determine benomyl resistance of *Botrytis* spp. and *Penicillium* spp., isolates were placed on APDA containing 100 μg of benomyl per milliliter and evaluated for growth after 5 days at 20 C.

Fruits infected with *Botrytis* spp. or *Penicillium* spp. were taken from four packinghouses from February to April in 1982, 1983, and 1984. Each house was sampled at least twice per year, and isolations were made from about 50 fruits in each sample. Fruits were disinfested with 0.525% sodium hypochlorite, rinsed with sterile distilled water, and tissue from decayed margins was plated on APDA containing 100 μg of benomyl per milliliter. Fungal growth was evaluated after 5 days at 20 C.

RESULTS

Considerable variability in propagule populations was observed among the nine packinghouses. During November 1981, for example, the population of *Penicillium* spp. in air ranged from 0.3 to 8.3 colonies per plate and dump-water populations ranged from 33 to 550 propagules per milliliter (Table 1).

Only two packinghouses operated weekly from September 1981 through April 1982. In these houses, spores of *Penicillium* spp. were more abundant in air and dump water than spores of *Botrytis* spp. or *M. piriformis* (Table 2). Airborne spores of *Penicillium* spp. increased significantly ($P = 0.05$) as the packing season progressed ($r = 0.93$). *Penicillium* spp. spores in dump water also were positively correlated with time ($P = 0.05$, $r = 0.80$). No significant trends were observed for *Botrytis* spp. or *M. piriformis* in air or dump water.

In 1982–1983, six packinghouses operated regularly from October to March, and monthly samples showed that populations of *Penicillium* spp. in dump water and air increased significantly ($P = 0.05$, $r = 0.90$ and 0.85, respectively) from October to March (Table 2). *Botrytis* spp. levels in dump water and air and *M. piriformis* in air were too low to determine any trends. *M. piriformis* in dump water increased significantly ($P = 0.05$, $r = 0.95$) from October to February (Table 2).

Six packinghouses used chlorine and three used SOPP for pears. No fungicides were used in dump water for apples. Average *M. piriformis* populations were 34, 142, and 142 per milliliter in 1981–1982 and 7, 292, and 387 per milliliter in 1982–1983 for dump water containing chlorine, SOPP, and no fungicide, respectively. Average *Penicillium* spp. populations were 534, 440, and 1,423 per milliliter in 1981–1982 and 162, 792, and 1,729 per milliliter in 1982–1983 for dump water containing chlorine, SOPP, and no fungicide, respectively. Thus, both chlorine and SOPP reduced *Penicillium* spp., but SOPP was relatively ineffective for reducing *M. piriformis* levels.

Table 1. Populations of *Penicillium* spp. in air and dump-tank water of nine apple and pear packinghouses during November 1981

Packinghouse	Air[a] (no. of colonies per plate)	Dump water[b] (no. of propagules per milliliter)
1	0.3	422
2	1.2	225
3	2.2	133
4	0.6	417
5	8.3	550
6	0.9	67
7	1.6	167
8	0.9	33
9	0.7	183

[a] Each value represents the mean number of colonies on three replicate 10-cm-diameter petri plates containing APDA per week exposed for 5 min in each of nine packinghouses.

[b] Each value represents the mean number of propagules per milliliter on three replicate plates per week from each packinghouse. Each dump-tank sample was diluted 1:99 with water, and 0.5 ml was placed on each plate of APDA.

Pathogenicity was similar in both years and data were combined. Sixty, 72, and 89% of *Botrytis* spp., *Penicillium* spp., and *M. piriformis* isolates, respectively, were pathogenic on pear fruit. Percentages of pathogenic *Penicillium* spp. isolates from air and dump water that were resistant to benomyl were similar and data were combined. Before December 1981, 4–8% of pathogenic *Penicillium* spp. isolates were resistant to benomyl, but from December to April, 15–31% of the isolates were resistant (Table 3). Although 87% of all benomyl-resistant *Penicillium* spp. isolates were less virulent than benomyl-sensitive *P. expansum* isolate 46, only 42% of the benomyl-sensitive isolates were less virulent than *P. expansum* isolate 46 on the basis of comparative lesion sizes of infected pear fruits. Because of the small number of *Botrytis* spp. isolates recovered, no monthly trends in benomyl resistance or virulence could be determined. Percentages of pathogenic *Botrytis* spp. isolates resistant to benomyl were less prevalent in air, water, and fruit than for *Penicillium* spp. isolates (Table 4). The percentages of benomyl-resistant *Botrytis* spp. and *Penicillium* spp. isolates from fruit exceeded those from air and water (Table 4).

DISCUSSION

Several species of *Penicillium* have been reported as pathogens of apple and pear, and *P. expansum* is more prevalent and more virulent than other species (8). Conway (9) isolated five species of *Penicillium* from apple dump-tank water, but only *P. expansum* was pathogenic. Only 28% of the *Penicillium* spp. collected from air and dump water in this study were nonpathogenic. Forty percent of *Botrytis* spp. isolates obtained in this study were nonpathogenic. Although *B. cinerea* has a wide host range and is an important pathogen of apple and pear (13), many species of *Botrytis* have a more restricted host range (12) and probably are carried into packinghouses in debris on fruit and bins.

Botrytis spp., *Penicillium* spp., and *M. piriformis* populations varied considerably between packinghouses and with time of year. Blanpied and Purnasiri (6) reported *P. expansum* populations in apple dump water from 300 to 28,000 propagules per milliliter. Bertrand and Saulie-Carter (4) reported a wide range in *M. piriformis* and *P. expansum* propagule levels in dump water from Oregon packinghouses. Factors such as amount of fruit and bin contamination, volume of fruit processed, and use of fungicide affect pathogen populations in dump water.

Penicillium spp. and *M. piriformis* spore levels increased as the packing season progressed, and increases were largest after November. Before December, most fungal propagules probably originated from orchard debris carried into packinghouses on bins. During December and until the end of the packing season, fungal spores probably came from decaying fruit stored in bins that passed through the dump water during the packing operation. This latter source of contamination appears to provide a greater number of spores than orchard sources. Although gray mold, caused by *B. cinerea*, is common in fruit stored for only 1–2 mo, sporulation on fruit is minimal because of absence of light (12). Thus, numbers of *Botrytis* spp. spores in dump water remained low throughout the packing season. Conversely, *M. piriformis* and *P. expansum* sporulate well on the surface of infected fruit in the dark.

The percentage of *Penicillium* spp. isolates resistant to benomyl was higher in packinghouse air and dump water from December through April than from

Table 2. Populations of *Botrytis* spp., *Penicillium* spp., and *Mucor piriformis* in packinghouse air and dump-tank water in 1981–1982 and 1982–1983

	Botrytis spp.				*Penicillium* spp.				*M. piriformis*			
	Air[a]		Dump water[b]		Air[a]		Dump water[b]		Air[a]		Dump water[b]	
Month	1981–1982	1982–1983	1981–1982	1982–1983	1981–1982	1982–1983	1981–1982	1982–1983	1981–1982	1982–1983	1981–1982	1982–1983
September	0.1	···	···	···	1.7	···	···	···	0.0	···	···	···
October	0.2	0.1	0	0	0.8	2.4	56	189	0.0	0.0	67	0
November	0.0	0.0	8	0	4.5	2.3	483	111	0.0	0.0	0	22
December	0.0	0.1	0	0	2.4	3.4	585	793	0.0	0.0	0	107
January	0.0	0.0	0	11	8.1	2.5	792	650	0.0	0.0	42	383
February	0.0	0.0	100	0	12.5	10.8	750	880	0.0	0.3	25	427
March	0.0	0.2	0	0	13.4	11.0	600	4,167	0.3	0.0	100	233
April	0.0	···	13	···	32.0	···	1,811	···	0.1	···	67	···

[a] Each value represents the average number of colonies on 10-cm-diameter petri plates containing APDA. Two packinghouses were sampled weekly in 1981–1982 and six were sampled monthly in 1982–1983. In each packinghouse, three replicate plates were exposed for 5 min.

[b] Each value represents the average number of propagules per milliliter in dump water from two packinghouses sampled weekly in 1981–1982 and from six packinghouses sampled monthly in 1982–1983. In each packinghouse, the dump-tank sample was diluted 1:99 with water, and 0.5 ml was placed on three replicate plates containing APDA.

Table 3. Benomyl resistance of pathogenic *Penicillium* spp. isolates from packinghouse air and dump tank water in 1981–1982

Month	No. of isolates tested[a]	Percent benomyl-resistant
September	149	4
October	115	7
November	153	8
December	163	20
January	125	26
February	112	15
March	161	24
April	116	31

[a] Isolates collected weekly from nine packinghouses and tested for pathogenicity to pear fruit and growth on APDA containing 100 μg of benomyl per milliliter.

Table 4. Benomyl resistance of pathogenic *Botrytis* spp. and *Penicillium* spp. from packinghouse air and dump-tank water and from infected Anjou pear fruit

	Percentage of isolates resistant to benomyl[a]			
	Air and dump water		Fruit	
Packing season	*Botrytis* spp.[b]	*Penicillium* spp.[c]	*Botrytis* spp.[d]	*Penicillium* spp.[e]
1981–1982	6	17	16	37
1982–1983	0	23	15	47
1983–1984	...	...	35	88

[a] Isolates tested for growth on APDA containing 100 μg of benomyl per milliliter.
[b] Values based on 67 and six isolates in 1982 and 1983, respectively, from nine packinghouses.
[c] Values based on 1,094 and 194 isolates in 1982 and 1983, respectively, from nine packinghouses.
[d] Values based on 122, 68, and 121 fruits in 1982, 1983, and 1984, respectively, from four packinghouses.
[e] Values based on 109, 53, and 65 fruits in 1982, 1983, and 1984, respectively, from four packinghouses.

September to November. Early-season isolates from orchard debris had low benomyl selection because preharvest use of benomyl is not recommended in the Mid-Columbia region. Later in the packing season, isolates originated from decaying fruit that was treated with benomyl and stored before packing. This selection pressure is especially evident in the high level of benomyl resistance of *Penicillium* spp. from decayed fruit stored at −1.1 C until February or April. Gutter et al (11) observed that the incidence of *P. digitatum* and *P. italicum* strains resistant to benomyl was low in orchards, medium in packinghouses, and high in storage rooms.

Bertrand (3) found that 33% of the *P. expansum* and 3% of *B. cinerea* isolates were resistant to benomyl at 100 μg/ml in both the 1975–1976 and 1976–1977 seasons. These isolates were from both dump water and infected fruit. When air, dump water, and fruit isolates of *Penicillium* spp. were combined in 1981–1982 and 1982–1983, benomyl resistance was 19 and 28%, respectively. Thus, the percentage of *Penicillium* spp. isolates resistant to benomyl in the Mid-Columbia region has not increased during the past 5 yr. The reason for this lack of increase is probably that benomyl has not been used in apple or pear orchards in the Mid-Columbia region, and its use on stone fruits is limited. It is also interesting that benomyl-resistant *Penicillium* spp. isolates were less virulent than benomyl-sensitive isolates. This agrees with a previous study in Oregon (3).

Both chlorine and SOPP appear to reduce *Penicillium* spp. levels in dump water. Chlorine reduced germination of *M. piriformis* sporangiospores and *P. expansum* conidia (16). SOPP was ineffective for reducing *M. piriformis* populations, and previous work supports this observation (14).

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