# Postharvest Decay of Winter Pear and Apple Fruit Caused by Species of *Penicillium*

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## ABSTRACT

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In surveys conducted during two fruit packing seasons, *P. expansum* and *P. solitum* were recovered most frequently (65 and 96% of collections, respectively, in 1990–91, and 77 and 54% of collections, respectively, in 1991–92) and in highest densities (1,893 cfu/ml and 749 cfu/ml, respectively, in 1990–91, and 308 cfu/ml and 667 cfu/ml, respectively, in 1991–92) from pear and apple dump tank water. *Penicillium solitum* was collected most frequently (89% of collections) and in greatest concentrations (1,634 cfu/ml) from drench solutions followed by *P. expansum* and *P. commune* (47 and 42% of collections, respectively, and 495 cfu/ml and 249 cfu/ml, respectively). *Penicillium expansum* was recovered from about 27% of field bins sampled while *P. solitum* and *P. commune* were recovered from about 10 and 8%, respectively. *Penicillium expansum* and *P. solitum* were the *Penicillium* spp. most frequently recovered from fruit sampled from packinghouses and markets. Pear fruit were treated with $2 \times 10^2$ conidia per milliter of *P. expansum* in challenge inoculations 0, 1, 7, or 28 days following initial treatment with $2 \times 10^3$ conidia per milliter of either *P. solitum* or *P. commune* to determine the competitive ability of *P. expansum* relative to these species. When incubated at 20 C for 7 days immediately following the challenge inoculation, *P. expansum* became established in wounds 0, 1, or 7 days following initial treatment with *P. solitum* and in water controls. *Penicillium expansum* did not become established in wounds challenged 28 days after initial treatments or in wounds challenged 7 days after initial treatment with *P. commune*. Incidence of infection by *P. expansum* following challenge inoculations 28 days after initial treatment with *P. solitum* was greater than in water controls when fruit were incubated at −1 C for 28 days followed by a ripening period of 7 days at 20 C. Incidence of infection by *P. expansum* generally decreased with increasing periods of time at which fruit were inoculated following initial wounding. Of 12 *Penicillium* spp. tested, only *P. aurantiogriseum, P. commune, P. crustosum, P. expansum, P. griseofulvum,* and *P. solitum* produced lesions in wounds of newly harvested, mature pear fruit (cv. d'Anjou).

Blue mold is one of the most important diseases of pome fruit in storage, shipping, and in the market (4,5,11,12,20). Although a large number of *Penicillium* spp. have been implicated as causal agents of postharvest decay of pome fruit (e.g., 1,3,11-13), blue mold is usually attributed to infection by *P. expansum* Link ex Grey. However, because it is difficult to accurately identify different *Penicillium* spp., the extent to which species other than *P. expansum* contribute to postharvest losses is unclear. In Australia, for example, *P. verrucosum* is considered a major component in blue-mold decay in New South Wales, (16), and *P. solitum* Westling was cited as accounting for more than 50% of blue mold in Victoria (12).

Species of *Penicillium* are ubiquitous soil inhabitants (7,17). Although some propagules of the various *Penicillium* spp. probably are transmitted to fruit surfaces by wind, it is likely that the majority of propagules are carried in soil and debris on field bins and equipment, and are transmitted to infection courts via water handling systems (10). Water systems are used to float pear and apple fruit from field bins to minimize mechanical damage to the fruit. In addition, prior to storage, pear and apple fruit are often drenched with ethoxyquin or diphenylamine, respectively, along with a fungicide (usually thiabendazole [TBZ]), to prevent superficial scald (28).

For the past several years, we have studied the role of *Penicillium* spp. other than *P. expansum* as causal agents of blue mold. The purposes of this study were to determine the diversity and density of *Penicillium* spp. in the postharvest environment of pome fruit in the Mid-Columbia region of Oregon and Washington, to examine the competitive abilities of the species that appeared to be prevalent in that environment and possible interactions between those species, and to determine which *Penicillium* spp. are indeed pathogenic to pear fruit. A preliminary report has been published (22).

## MATERIALS AND METHODS

**Surveys.** Flotation water in commercial pear and apple packinghouse dump tanks (the receiving portion of fruit sorting and packing lines), prestorage drench solutions, field bins, and fruit were sampled periodically in 1991 and 1992 to determine the diversity and density of *Penicillium* spp. in the postharvest environment. Samples were collected from up to four commercial packinghouses (three in the Hood River Valley, OR, and one in White Salmon, WA) depending on whether or not fruit was being packed on that day.

*Dump tanks.* From mid-February to mid-April 1991, dump tank water from all four packinghouses was sampled at approximately weekly intervals. Water samples were taken close to where fruit containing field bins were immersed into the tanks. Single 0.1-ml aliquots of the flotation water were removed from the tanks with a sterile pipette and transferred into test tubes containing 9.9 ml of sterile distilled water ($10^{-2}$ dilution). Three 0.2-ml subsamples were spread onto Czapek's Yeast Agar (CYA; 17), a medium that accentuates morphological differences among *Penicillium* spp., amended with 200 ppm of chloramphenicol (CYAC) in petri dishes. Cultures were incubated on a laboratory bench in clear plastic refrigerator boxes at room temperature (21 ± 2 C). After 7 days, all colonies resembling *Penicillium* were

grouped by surface and reverse colony color and morphology. Questionable isolates were examined under a light microscope to verify their identy as *Penicillium* spp. Representatives of each isolate type were transferred singly to dishes of CYA and incubated at room temperature. These colonies were compared after 7 days and representative isolates of each type were transferred to slant tubes containing either potato-dextrose agar (PDA; Difco Laboratories, Detroit, MI) or CYA for storage at 4 C.

In the 1991–92 packing season, dump tank water samples were collected from the Hood River Valley packinghouses only. Water samples were collected and treated as described above except that two subsamples were cultured from each of three samples collected at each site.

*Drenches.* During the 1991 harvest period, pear drench solutions containing ethoxyquin and TBZ were sampled from two packinghouses in the Hood River Valley and one from Yakima, Washington. Samples were collected either from runoff when a load of fruit was being treated or directly from the drench solution holding tank. Samples were collected and treated as described above using two subsamples from each of three samples taken at each site.

*Field bins.* Samples were taken from wooden field bins on 8 August 1991, about 2 wk before the start of the Bartlett pear harvest, to determine if the bins were infested with decay fungi. Composite samples from each bin were made by scraping the surfaces of three areas, each approximately 1 $cm^2$, with a sterile scalpel to remove the surface layer of wood and accompanying debris. The scrapings were transferred to screw cap bottles containing 10 ml of sterile distilled water. Sample sites were selected from areas on the bin that looked particularly stained or had traces of fruit remaining from the previous season. Scrapings were taken from each of three bins at each of 16 sampling sites scattered around the Hood River Valley. Two subsamples from each sample were plated on CYAC and treated as described above.

*Fruit.* To determine if *Penicillium* spp. other than *P. expansum* contribute to fruit decay in storage, apple and pear fruit with blue-mold lesions were collected each time that flotation water samples were taken. Ten to 12 fruit of each cultivar being processed at the time of sampling were collected from cull bins. In 1990–91, fruit were selected for typical blue mold symptoms. In 1991–92, fruit with less typical symptoms of blue mold or with symptoms similar to bull's-eye rot, Alternaria rot, or Cladosporium rot (caused by *Pezicula malicorticis* (H. Jacks.) Nannf., *Alternaria alternata* (Fr.:Fr.) Keissl., and *Cladosporium herbarum* (Pers.:Fr.) Link, respectively) were selected. Fruit were placed either in plastic bags or clear plastic refrigerator boxes and brought to the laboratory where decay organisms were isolated from them.

All fruit were surface disinfested prior to isolation of decay fungi. Fruit with lesions covered with large masses of spores first were wetted with 95% ethanol then dipped in 0.05% NaOCl for about 5 min. Other fruit were either dipped in 0.05% NaOCl for about 5 min or wiped with tissue paper saturated with 95% ethanol and allowed to dry under the laminar flow hood. Two sections (approx. 16 $mm^3$) were removed from the margin of each lesion beneath the fruit epidermis and placed in petri dishes containing CYA. Cultures were incubated and treated as described above.

During the 1991–92 packing season, fruit also were collected from local markets. Produce managers and personnel were asked to collect all apple and pear fruit that were culled for decay either at delivery or from the grocery shelves. Isolations from all decayed fruit were done as described above.

**Identification of isolates.** Type isolates were identified to species with zymograms produced by electrophoresis of extracellular pectic and amylolytic isoenzymes in polyacrylamide gels (6). To prepare cultures for zymogram analysis, isolates were grown in liquid media containing either pectin or wheat kernels as their carbon sources for 7 days at room temperature. After 7 days, culture media were separated from mycelia and the media were stored in polypropylene micro-centrifuge tubes (VWR Scientific, Portland, OR) at −20 C until used for analysis. A total of 397 type isolates were screened.

**Species competition.** To determine why so much of the decay observed in the commercial cold storages appeared to be caused by *P. expansum* even though other pathogenic species were present in relatively large numbers in the postharvest dump tank and drench solutions, a series of inoculations using combinations of *Penicillium* spp. with *P. expansum* was conducted. In these experiments, wounds on pear fruit (cv. d'Anjou) initially were inoculated with *P. solitum* (isolate HR-126) or *P. commune* Thom (HR-4). These inoculations were later challenged by inoculating the same wound with *P. expansum* (isolate HVL-99).

Inocula were prepared by flooding 7-day-old colonies of the respective *Penicillium* spp., which were growing in slant tube cultures on PDA, with sterile distilled water. The surfaces of the colonies were gently rubbed with a sterile rubber policeman and 0.1 ml of the resulting spore suspension was transferred to another test tube containing about 10 ml of sterile distilled water. This suspension was vigorously mixed using a vortex mixer for 1 min, sonicated for 3 min (Model T14, L&R Manufacturing Co., Kearny, NJ), and mixed again with the vortex mixer for 1 min to break up chains of conidia and assure uniform distribution. Density of conidia was determined using a hemacytometer. Inoculum concentrations were adjusted to $2 \times 10^3$ spores per milliter of *P. solitum* and *P. commune*, and $2 \times 10^2$ spores per milliter of *P. expansum*.

Fruit used in these experiments and those described below were harvested from mature d'Anjou pear trees growing at the Mid-Columbia Agricultural Research and Extension Center. Fruit were picked at commercial harvest when pressures were 58–67 N (9). No fungicides were applied to the trees or fruit during the growing season.

Fruit were surface disinfested before inoculation by soaking them in a tub containing approx. 0.05% NaOCl for at least 5 min. Fruit then were placed in ventilated polyethylene bags within wooden fruit boxes that had been similarly disinfested. Fruit were stored for 1 or 2 days at −1 C in a cold room before they were inoculated.

Postharvest decay pathogens of pome fruit are typically wound parasites; therefore, fruit were wounded prior to inoculation. Two puncture wounds (6 mm diam × 3 mm deep) were made on the face of each of three fruit per replicate with three replicates per treatment. Each wound then was inoculated with 50 µl of either *P. solitum* or *P. commune* conidia suspensions or sterile distilled water. These initial treatments were followed (challenged) by inoculating the same wounds with either 50 µl of a suspension of conidia of *P. expansum* or sterile distilled water. Challenge inoculations were made either immediately, or 1, 7, or 28 days after initial treatments. Fruit were placed on cardboard fruit trays in standard corrugated cardboard fruit boxes lined with vented polyethylene bags. Fruit were returned to −1 C cold storage within 4 h after each initial inoculation. The experiment was a completely randomized design; however, fruit inoculated at each challenge interval were kept separated in individual boxes to avoid contamination.

Two sets of fruit were inoculated at each challenge interval. The first set was placed in a growth chamber at 20 C immediately after the challenge inoculation to allow the pathogens to incubate while fruit ripened. Fruit were assessed for disease incidence 7 days later, at which time isolations were made from the margins of lesions to determine the *Penicillium* spp. present. Isolations were made as described above. The second group was stored for 4 wk at −1 C after challenge inoculations with *P. expansum*, at which time they were assessed for disease incidence and then placed at 20 C for 7 days. After the ripening period, disease incidence again was assessed and isolations made from the margins of lesions to determine the identity of the *Penicillium* spp. present. In addition, five freshly wounded, surface-disinfested fruit were inoculated with *P. expansum*, as described above, at each challenge time to check the virulence of the inoculum. These fruit were placed on cardboard fruit trays in lidded, clear plastic refrigerator boxes. Boxes were placed in an incubator at 20 C for 7 days, at which time disease incidence was assessed. The experiment was conducted twice.

**Effect of temperature on growth of *Penicillium* spp. in vivo.** Growth rates of isolates of *P. expansum* (HVL-99), *P. solitum* (HR-126), and *P. commune* (HR-4) in pear fruit (cv. d'Anjou) at −1, 4, 8, and 12 C were examined. Fruit were surface disinfested, wounded, and inoculated as described above. Inocula were prepared as described above and adjusted to $2 \times 10^3$ conidia per milliter. Each of four replicates of five fruit with two wounds per fruit was placed in an individual cardboard fruit box. Fruit inoculated with each species were placed in separate boxes to prevent cross contamination. Colony diameters were assessed every 7 days for up to 28 days. Fruit that were to be assessed at each time period were randomly arranged within each box. The fruit assessed each week were not replaced in the boxes to further avoid contamination. The experiment was conducted twice.

Rates of decay caused by each species at each temperature were determined by regression analysis. For each species, the slopes and intercepts of each of the lines determined for each temperature were compared.

**Pathogenicity of *Penicillium* spp.** To determine pathogenicity of *Penicillium* spp. previously reported as pathogenic on pome fruit (1,3,8,11–13,15,16,18,19,21,22,27,28), pear fruit (cv. d'Anjou) were inoculated with isolates whose identity had been verified by zymograms. Two isolates each were used of *P. aurantiogriseum* (92-15 and 92-27, Hood River, OR), *P. brevicompactum* (R85-73, R. Roberts, USDA-ARS, Wenatchee, WA; 91-90, Hood River, OR), *P. chrysogenum* (91-48 and 91-117, Hood River, OR), *P. commune* (91-38, Hood River, OR; R89-19, R. Roberts, USDA-ARS, Wenatchee, WA), P. crustosum (ATTC-10430, American Type Culture Collection; HR-768, WA), *P. expansum* (91-79 and 92-47, Hood River, OR) *P. roquefortii* (91-12 and 91-175, Hood River, OR), *P. solitum* (91-13 and 91-14, Hood River, OR), *P. verrucosum* (91-37, Hood River, OR; ATTC-48957, American Type Culture Collection), and *P. rugulosum* (91-110 and 91-118, Hood River, OR); single isolates were used of *P. digitatum* (92-26, Hood River, OR) and *P. griseofulvum* (91-150, Yakima, WA). Where possible, isolates originally recovered from lesions on fruit were used. Isolates 92-15 and 92-27 were originally from apple fruit. Isolates 91-12, 91-13, 91-14, 91-38, 91-48, 91-117, 91-175, 92-26, and 92-47 were from pear fruit; 91-79 was originally isolated from a cherry fruit. Isolates 91-90, 91-117, 91-110, 91-118, and 91-150 were originally recovered from drench solutions; HR-768 and 91-37 were recovered from pear flotation water.

Inocula were prepared as described above. The concentration of inoculum of each isolate was adjusted to $2 \times 10^3$ conidia per milliter. Fruit were surface disinfested, wounded, and inoculated as described above. Two wounds on each of five fruit were inoculated with each isolate, each wound constituting a single replicate. Water-inoculated fruit were included as negative controls.

Fruit were randomly arranged on cardboard fruit trays such that one fruit inoculated with each isolate was on each tray. Trays were placed in standard cardboard fruit boxes lined with ventilated polyethylene bags. Two sets of fruit were inoculated, one of which was placed in an incubator at 20 C for 7 days and the other in cold storage at −1 C for 16 wk.

Wounds were assessed for lesion development (incidence) at the end of the respective incubation periods. Fruit with wounds in which lesions had not developed by the end of the cold storage treatment were placed at 20 C for 7 days to assess disease development during ripening. Isolations were made from all fruit showing symptoms of decay to complete Koch's postulates for determining pathogenicity.

Viability of conidia used for inocula also was assessed. Four 50-μl droplets each of the concentrated aqueous suspension used to make inoculum of each isolate were placed on CYA in petri dishes. Dishes were incubated at room temperature for 24 h, at which time germination was assessed. A drop of aniline blue in lactophenol was placed at the location at which one of the inoculum droplets had been placed earlier. Germinated and ungerminated conidia were counted under a light microscope at 250×. All conidia in a single continuous traverse of the dish were counted until 100 conidia had been assessed. Conidia were considered germinated if germ tubes were at least one conidium diameter in length.

**Data analysis.** All proportions were transformed to arcsine square root values (23) prior to analyses. Statistical analyses were conducted with MINITAB statistical software, release 7.2 (Minitab Inc., State College, PA). Data from each trial were combined and subjected to analysis of variance with trial as a block. Denominator terms for calculating *F*-statistics were the pooled sums of squares for all trial × treatment interactions divided by their respective degrees of freedom. Treatment means were separated by Fisher's protected least significant difference with $P = 0.05$.

## RESULTS

**Surveys.** *Dump tanks.* In both years, the *Penicillium* spp. most frequently recovered from dump tank solutions were *P. expansum* and *P. solitum* (Table 1). Of the six *Penicillium* spp. identified in 1990–91, *P. solitum* was collected most frequently, followed by *P. expansum*. At those times when *P. expansum* was collected, however, propagule densities of *P. expansum* were over two and one-half times greater than those of *P. solitum*. In the 1991–92 fruit packing season, *P. expansum* was found most frequently, followed by *P. solitum*. Twice as many propagules of *P. solitum* were recovered as of *P. expansum*. The numbers of propagules of both species recovered were less than in the previous year.

*Drenches.* At least 12 *Penicillium* spp. were recovered from drench solutions (Table 2). *P. solitum* was recovered most frequently, and in relatively high density. *P. expansum* was recovered from about 50% of the collections, and the mean spore density was less than one-third that of *P. solitum*. *P. commune* was recovered almost as often as *P. expansum* and about 38% more often than *P. roquefortii*.

TABLE 1. Composition of species of *Penicillium* recovered from dump tank flotation solutions in pear and apple packinghouses in the Mid-Columbia region of Oregon and Washington

| *Penicillium* spp. | Frequency[u] | Cfu/ml[v] Mean | ± SD[w] |
|---|---|---|---|
| 1990–91 packing season | ($n = 23$)[x] | | |
| *P. aurantiogriseum* | 4.3 | 83.3 | ... |
| *P. commune* | 21.7 | 158.3 | 18.6 |
| *P. expansum* | 65.2 | 1,893.0 | 2,031.0 |
| *P. roquefortii* | 26.1 | 208.3 | 102.1 |
| *P. solitum* | 95.6 | 749.0 | 661.0 |
| *P. verrucosum* | 4.3 | 83.3 | ... |
| *P.* spp.[y] | 4.3 | 166.7 | ... |
| Other[z] | 34.8 | 599.0 | 503.0 |
| 1991–92 packing season | ($n = 13$)[x] | | |
| *P. commune* | 23.1 | 306.0 | 316.0 |
| *P. expansum* | 76.9 | 308.0 | 439.0 |
| *P. glabrum* | 7.7 | 83.3 | ... |
| *P. roquefortii* | 23.1 | 138.9 | 96.2 |
| *P. rugulosum* | 15.4 | 83.3 | 0.0 |
| *P. solitum* | 53.8 | 667.0 | 771.0 |
| *P. verrucosum* | 7.7 | 83.3 | ... |
| *P.* spp.[y] | 23.1 | 111.1 | 48.1 |
| Other[z] | 7.7 | 1,583.0 | ... |

[u] Percentage of collections in which a species was recovered.
[v] Determined from counts of colonies growing on plates of Czapek's Yeast Agar (CYA) + 200 ppm chloramphenicol or acidified CYA that had been inoculated with 0.2 ml of $10^{-2}$ dilutions of flotation solutions and incubated for 7 days at 20–23 C. In 1990–91, single samples (3 plates/sample) were taken at each collection, whereas in 1991–92, 3 samples (2 plates/sample) were taken.
[w] Standard deviation; where standard deviations are not given, isolates of a species were recovered at one collection only.
[x] Number of collections made during the packing season.
[y] Species of *Penicillium* whose positive identity was not determined.
[z] Colonies of *Penicillium* lost due to overgrowth by either *Mucor* or *Trichoderma* before they could be identified.

*Field bins.* Isolates of four genera of organisms pathogenic to pome fruit, including *Penicillium,* were recovered from the surfaces of wooden field bins (Table 3). Species of *Penicillium* were recovered at 75% of the locations where bins were sampled and from fewer than one-half of the total number of bins sampled. Of those species, *P. expansum* was recovered most frequently, followed by *P. solitum* (Table 3). *Penicillium commune* and *P. roquefortii* also were recovered.

*Fruit.* In the 1990–91 fruit packing season, both winter pear and apple fruit with decay lesions were collected from packinghouses. *Penicillium expansum* was isolated from almost all of the pear fruit and from all of the apple fruit (Table 4). In 1991–92, only winter pear fruit were collected from packinghouses. Although *P. expansum* was isolated from the majority of fruit (62%), *P. solitum* also was recovered from a relatively high percentage of fruit (27%).

Fruit collected from markets had been culled by various people, resulting in inconsistencies in the type of fruit culled. For example, a clerk in one market may have culled fruit from shipping boxes only and another clerk in the same or a different market may have taken fruit only from the shelf. In addition, much of the fruit culled from markets was senescent. Overall, *P. expansum* was recovered from about 80% and *P. solitum* from about 16% of the winter pears that were received (Table 4). In contrast, *P. expansum* and *P. solitum* were recovered from about 61% and 34%, respectively, of the apples received.

**Species competition.** Disease incidence, expressed as the percentage of wounds with lesions regardless of causal *Penicillium* spp. (total lesions), was differentially affected by both initial inoculation treatments and length of time from initial treatments to subsequent challenges. Disease incidence resulting from infection by *P. expansum* also was differentially affected by those factors. Significant interactions were observed both in fruit incubated at 20 C for 7 days immediately after being challenged by *P. expansum* ($P = 0.027$ for total lesions and $P < 0.001$ for those from which *P. expansum* was recovered) and in fruit incubated in cold storage (−1 C) for 28 days ($P = 0.008$ for total lesions and $P < 0.001$ for those from which *P. expansum* was recovered). All wounds on fruit inoculated with *P. expansum* to check challenge inoculum virulence became infected.

On fruit incubated at 20 C immediately following challenge inoculations, the total percentages of wounds that developed

TABLE 2. Composition of species of *Penicillium* in drench solutions for postharvest treatment of pear and apple fruit in the Mid-Columbia region of Oregon and Washington

| *Penicillium* spp. | Frequency[x] | Cfu/ml[y] Mean | ± SD[z] |
|---|---|---|---|
| *P. aurantiogriseum* | 5 | 83.3 | ... |
| *P. brevicompactum* | 16 | 125.0 | 41.7 |
| *P. chrysogenum* | 11 | 292.0 | 29.5 |
| *P. commune* | 42 | 249.0 | 305.0 |
| *P. corylophilum* | 11 | 1,675.0 | 2,227.0 |
| *P. expansum* | 47 | 495.0 | 496.0 |
| *P. fellutanum* | 5 | 500.0 | ... |
| *P. griseofulvum* | 5 | 1,000.0 | ... |
| *P. roquefortii* | 26 | 133.3 | 34.9 |
| *P. rugulosum* | 5 | 83.3 | ... |
| *P. simplicissimum* | 5 | 100.0 | ... |
| *P. solitum* | 89 | 1,634.0 | 3,334.0 |
| *P.* spp. | 32 | 227.8 | 211.0 |

[x] Percentage of collections ($n = 19$) in which a species was recovered.
[y] Determined from counts of colonies growing on plates of Czapek's Yeast Agar + 200 ppm chloramphenicol that had been inoculated with 0.2 ml of $10^{-2}$ dilutions of drench solutions and incubated for 7 days at 20–23 C.
[z] Standard deviation.

TABLE 3. Occurrence of genera of known pathogenic fungi and species of *Penicillium* recovered from wooden pear and apple field bins in the Hood River Valley prior to harvest in 1991

| Taxa | Frequency of recovery[z] Locations ($n = 16$) | Bins ($n = 48$) |
|---|---|---|
| *Alternaria* spp. | 100.0 | 68.8 |
| *Botrytis* spp. | 6.3 | 2.1 |
| *Cladosporium* spp. | 68.8 | 39.6 |
| All *Penicillium* spp. | 75.0 | 43.8 |
| *P. commune* | 25.0 | 8.0 |
| *P. expansum* | 75.0 | 27.0 |
| *P. roquefortii* | 25.0 | 8.0 |
| *P. solitum* | 31.0 | 10.0 |
| *P.* spp. | 31.0 | 10.0 |

[z] Frequency expressed as the percentage of sites (each of 3 bins at each of 16 locations) from which the fungi were recovered.

TABLE 4. Species of *Penicillium* isolated from decay lesions on winter pear and apple fruit collected from packinghouses and markets in the Mid-Columbia region of Oregon and Washington during the 1990–91 and 1991–92 fruit packing seasons

| | Fruit infected (%) | | | | |
|---|---|---|---|---|---|
| | Packinghouses | | Markets | | |
| | 1990–1991 | | 1991–1992 | | |
| *Penicillium* spp.[u] | Pear[v] | Apple[w] | Pear[x] | Pear[y] | Apple[z] |
| *P. aurantiogriseum* | 0 | 0 | 0 | 0 | 3.8 |
| *P. chrysogenum* | 0.5 | 0 | 0 | 0 | 0 |
| *P. commune* | 0.9 | 0 | 7.8 | 1.8 | 1.3 |
| *P. digitatum* | 0 | 0 | 0 | 1.8 | 0 |
| *P. expansum* | 92.9 | 100.0 | 62.3 | 80.7 | 61.3 |
| *P. roquefortii* | 0.5 | 0 | 2.6 | 0 | 0 |
| *P. solitum* | 3.8 | 0 | 27.3 | 15.8 | 33.8 |
| Other | 1.4 | 0 | 0 | 0 | 0 |
| Total number of fruit | 211 | 33 | 77 | 57 | 80 |

[u] Isolations made by placing two sections from the margins of each lesion on Czapek's Yeast Agar (CYA) or CYA + 200 ppm chloramphenicol.
[v] Cultivars included d'Anjou and Bosc.
[w] Cultivars included Red Delicious, Newtown, and Granny Smith.
[x] Cultivars included d'Anjou, Bosc, and Red Anjou.
[y] Cultivars included d'Anjou, Bosc, Comice, and Red Anjou.
[z] Cultivars included Golden Delicious, Red Delicious, Newtown, and Granny Smith.

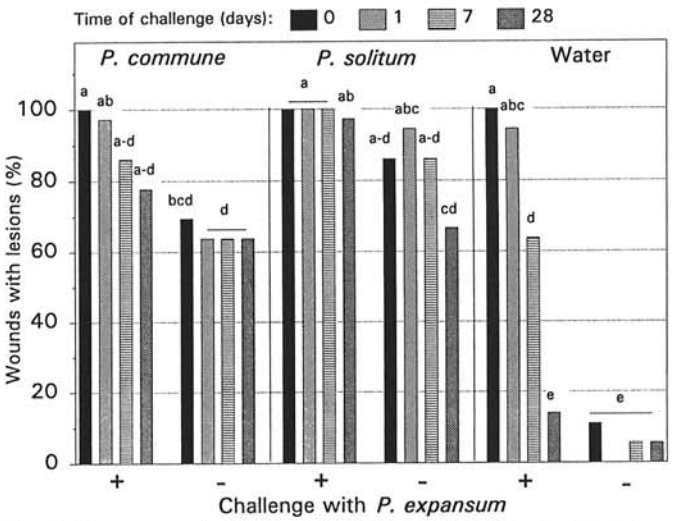


**Fig. 1.** Percentage of wounds in pear fruit (cv. d'Anjou) with lesions after incubation at 20 C for 7 days following challenge inoculations with aqueous spore suspensions of *Penicillium expansum* ($2 \times 10^2$ conidia per milliliter) 0, 1, 7, and 28 days after initial treatments with either *P. solitum* or *P. commune* ($2 \times 10^3$ conidia per milliliter, each), or with sterile water. Fruit were stored and incubated at −1 C after initial treatment. A significant interaction was observed between initial treatments and time of challenge ($P < 0.001$). Treatment means with common letters were not significantly different according to Fisher's protected LSD at $P = 0.05$.

lesions following challenges did not differ significantly within initial treatment groups except for the water control group (Fig. 1). In that group, significantly fewer lesions occurred with increasing length of time between initial wounding and the 7 and 28 day challenges. The percentage of wounds that developed lesions following the 28 day challenge was not significantly different from that observed in uninoculated controls. In groups challenged with *P. expansum*, lesions developed in about 78–100% of wounds initially inoculated with *P. commune* and 97–100% of wounds initially inoculated with *P. solitum*. Similar results were observed in fruit that was incubated at −1 C. However, the percentages of lesions that developed following the 7 and 28 day challenges with *P. expansum* in initial water treatments were not significantly different from those observed in uninoculated controls (Fig. 2). In addition, somewhat lower percentages of lesions occurred in unchallenged wounds initially inoculated with either *P. commune* or *P. solitum* than were observed in fruit incubated only at 20 C.

Initial inoculations with *P. commune* or *P. solitum* had no apparent effect on the percentage of wounds from which *P. expansum* was recovered following 0, 1, and 28 day challenges in fruit incubated at 20 C immediately after challenge inoculations. *Penicillium expansum* was recovered from >90% of wounds challenged 0 and 1 days after initial treatments, and significant differences were not observed among initial treatment classes corresponding with those challenge times (Fig. 3). In addition, the percentages of wounds from which *P. expansum* was recovered following the 28 day challenges were not significantly different from those observed in the unchallenged controls regardless of initial treatment. Following the 7-day challenge, however, different results were observed dependent on initial treatment. The percentage of wounds from which *P. expansum* was recovered following challenge inoculations 7 days after initial treatment with *P. solitum* (81%) was not significantly different from that resulting from the 0 and 1 day challenges. *Penicillium expansum* was recovered from somewhat fewer wounds that had been challenged 7 days following initial treatment with sterile water (64%). Following initial treatment with *P. commune*, however, the percentage of wounds from which *P. expansum* was recovered was not significantly different from that observed in unchallenged controls and was significantly less than the percentage from that in the water control (Fig. 3). *Penicillium expansum* was recovered from less than 15% of the wounds that had not been inoculated with it (Fig. 3).

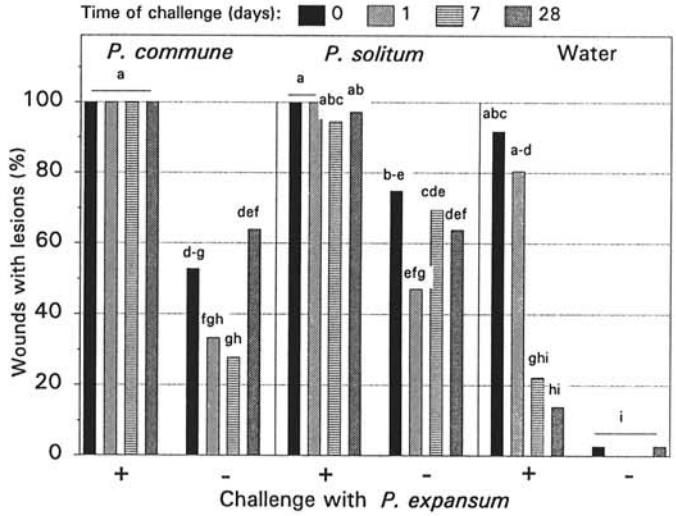


**Fig. 2.** Percentage of wounds on pear fruit (cv. d'Anjou) with lesions after incubation at −1 C for 28 days followed by 7 days at 20 C after challenge inoculations with aqueous spore suspensions of *Penicillium expansum* ($2 \times 10^2$ conidia per milliliter) 0, 1, 7, and 28 days after initial treatments with either *P. solitum* or *P. commune* ($2 \times 10^3$ conidia per milliliter, each), or with sterile water. Fruit were stored and incubated at −1 C after initial treatment. A significant interaction was observed between initial treatments and time of challenge ($P < 0.001$). Treatment means with common letters were not significantly different according to Fisher's protected LSD at $P = 0.05$.

Similarly, the incidence of infection by *P. expansum* after challenge inoculations 0 and 1 days after initial treatments was not significantly different regardless of initial treatment for fruit incubated at −1 C for 28 days (Fig. 4). *Penicillium expansum* was recovered from significantly fewer lesions following the 7 day challenge than from the 0 and 1 day challenges regardless of initial treatment; within initial treatments no further reduction in incidence of *P. expansum* following the 28 day challenge was observed. Furthermore, following the 28 day challenge, the incidence of *P. expansum* in wounds in which *P. solitum* was initially inoculated increased over that observed resulting from the 7 day

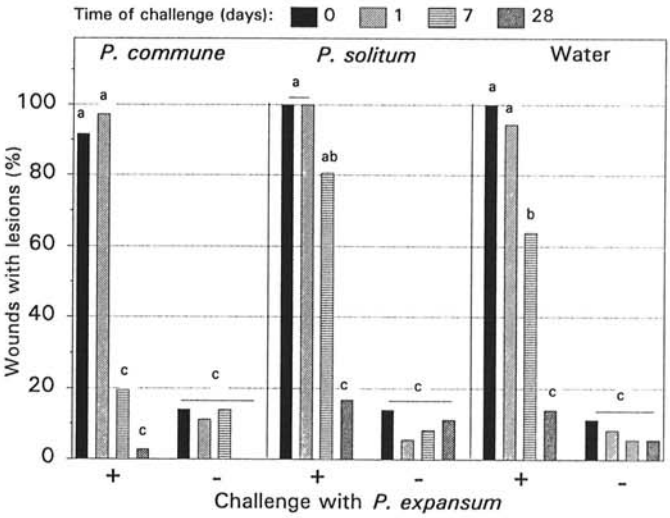


**Fig. 3.** Percentage of wounds in pear fruit (cv. d'Anjou) with lesions caused by *Penicillium expansum* after incubation at 20 C for 7 days following challenge inoculations with aqueous spore suspensions ($2 \times 10^2$ conidia per milliliter) 0, 1, 7, and 28 days after initial treatments with either *P. solitum* or *P. commune* ($2 \times 10^3$ conidia per milliliter, each), or with sterile water. Fruit were stored and incubated at −1 C after initial treatment. A significant interaction was observed between initial treatments and time of challenge ($P < 0.001$). Treatment means with common letters were not significantly different according to Fisher's protected LSD at $P = 0.05$.

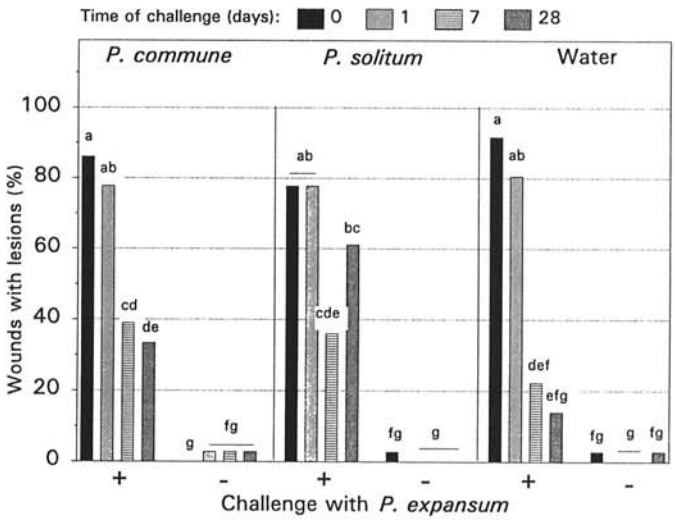


**Fig. 4.** Percentage of wounds on pear fruit (cv. d'Anjou) with lesions caused by *Penicillium expansum* after incubation at −1 C for 28 days followed by 7 days at 20 C after challenge inoculations with aqueous spore suspensions ($2 \times 10^2$ conidia per milliliter) 0, 1, 7, and 28 days after initial treatments with either *P. solitum* or *P. commune* ($2 \times 10^3$ conidia per milliliter, each), or with sterile water. Fruit were stored and incubated at −1 C after initial treatment. A significant interaction was observed between initial treatments and time of challenge ($P < 0.001$). Treatment means with common letters were not significantly different according to Fisher's protected LSD at $P = 0.05$.

challenge and was not significantly different from the challenges made at 0 and 1 days after initial treatment.

**Effect of temperature on growth of *Penicillium* spp. in vivo.** Results differed between trials; therefore, the treatment means from both trials were used for the final analyses. Slopes of lines describing growth of each species at each temperature were significantly different for each species (Fig. 5). All lines at 4, 8, and 12 C had slopes significantly greater than 0; however, no growth was observed by any species at −1 C during the experiments. *Penicillium expansum* grew fastest at 4, 8, and 12 C, followed by *P. solitum*, then *P. commune*. Intercepts of lines at each temperature for each species were not significantly different.

**Pathogenicity of *Penicillium* spp.** Seven of the 12 *Penicillium* spp. produced lesions in wounds of pear fruit. Only *P. expansum, P. crustosum, P. solitum, P. commune, P. griseofulvum, P. verrucosum,* and *P. aurantiogriseum* were pathogenic on freshly harvested d'Anjou fruit after 7 days at 20 C (Table 5). Differences in virulence, in terms of numbers of wounds infected and lesion diameters, were observed among species. Differences were consistent among trials but varied among incubation regimes. When incubated at 20 C, *P. expansum* and *P. crustosum* were the most virulent species, producing lesions in all wounds into which they were inoculated (Table 5). Lesions caused by these species also were consistently larger than those produced by other species. *Penicillium solitum* produced more and larger lesions than did *P. commune* ($P = 0.05$). *Penicillium aurantiogriseum* consistently produced a few small lesions, whereas *P. griseofulvum* produced lesions only in the second trial and no lesions developed in wounds inoculated with *P. verrucosum*. Viability of conidia of each species used for inoculum was greater than 90%. Consistent with results observed at 20 C, *P. expansum* was the most virulent species at −1 C in terms of both disease incidence and severity. In general, however, both incidence and severity of disease were greatly reduced in fruit kept in cold storage as opposed to being ripened immediately after inoculation. Virulence of *P. crustosum* was reduced to levels equivalent to those of *P. solitum* and *P. commune* in low temperature storage. Neither *P. aurantiogriseum* nor *P. griseofulvum* produced lesions in fruit incubated at −1 C.

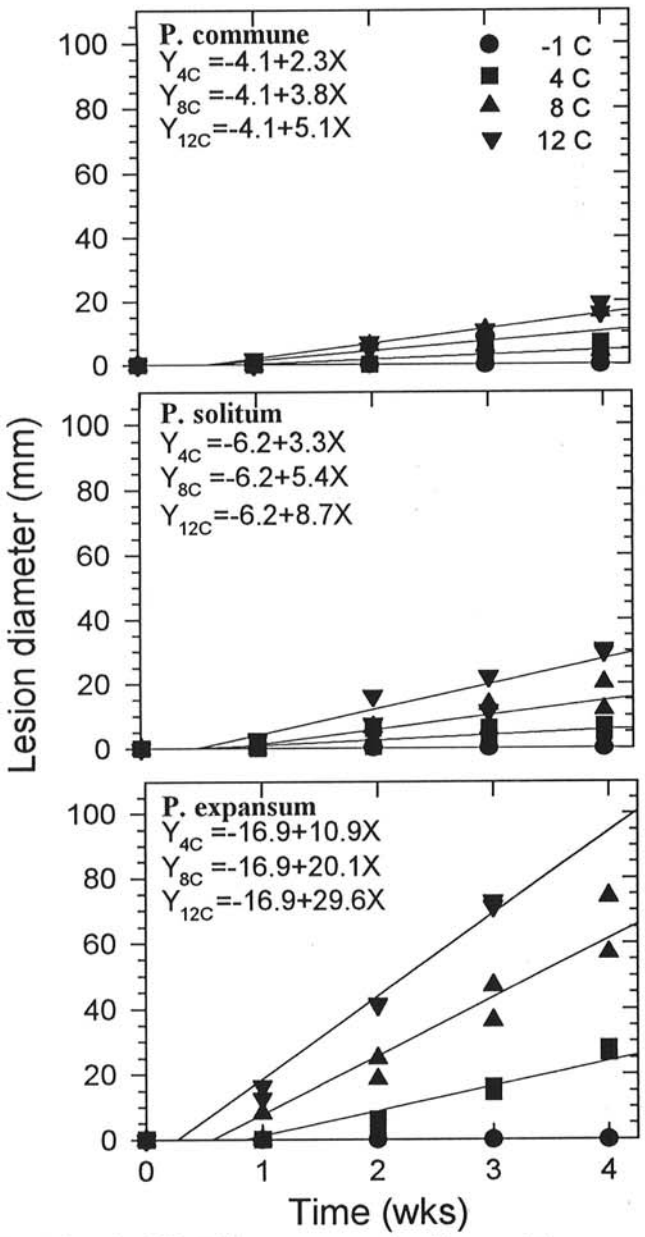


**Fig. 5.** Growth of *Penicillium expansum, P. solitum,* and *P. commune* on mature pear fruit (cv. d'Anjou) at −1, 4, 8, and 12 C. Fruit were puncture-wounded (6 mm diam. × 3 mm deep) and inoculated with 2 × $10^3$ conidia per milliliter.

## DISCUSSION

Diversity of *Penicillium* spp. was relatively high in water systems (i.e., dump tanks and drenches) and field bins in the postharvest environment of pears and apples in the Mid-Columbia region compared with that reported previously from other regions. *Penicillium expansum* and *P. solitum* were found most frequently and in greatest abundance. In New York, about 80% of isolates collected from apple water handling systems were identified as *P. aurantiogriseum,* and nearly all of the remaining 20% were *P. expansum* (21). In that case, however, the authors suspected that *P. aurantiogriseum* would prove to be *P. solitum* based on zymograms, which would be in agreement with our findings. In contrast, *P. expansum* was recovered almost exclusively in packing shed water systems in Victoria, Australia (26). The lack of diversity observed in Victoria compared with that in the Mid-Columbia may be due to differences in sampling methods.

Almost twice as many *Penicillium* spp. were recovered from drench solutions as from dump tank water, despite the fact that the drench solutions contained TBZ. The reason for this is not readily apparent. Although some loss of species could occur in storage, the dump tanks sampled were from both presize and packing lines and both stored and unstored fruit were being processed at different sampling times. Almost all dump tanks were treated with either NaOCl or sodium-ortho-phenylphenate (SOPP). In general, those tanks treated with chlorine contained fewer propagules of *Penicillium* than did those treated with SOPP. It may be that SOPP affects different *Penicillium* spp. differentially or that either of the treatments eliminated enough of

TABLE 5. Incidence and severity of disease caused by species of *Penicillium* inoculated onto pear fruit (cv. d'Anjou) after incubation at 20 C for 7 days or −1 C for 16 wk[v]

| | 20 C | | −1 C | |
|---|---|---|---|---|
| *Penicillium* spp. | Incidence (%)[w] | Severity (mm)[x] | Incidence (%) | Severity (mm) |
| *P. aurantiogriseum* | 7.5 d[y] | 6.0 c | —[z] | — |
| *P. commune* | 47.5 bc | 6.9 c | 40.0 ab | 11.1 b |
| *P. crustosum* | 100.0 a | 20.1 b | 20.0 b | 9.8 b |
| *P. expansum* | 100.0 a | 37.2 a | 87.5 a | 48.3 a |
| *P. griseofulvum* | 20.0 cd | 4.3 c | — | — |
| *P. solitum* | 73.3 b | 9.0 c | 17.5 b | 12.9 b |
| *P. verrucosum* | — | — | 7.5 b | 9.3 b |

[v] Results of two trials combined. In each trial, two 6 mm diam × 3 mm deep puncture wounds were made on each of five fruit. Wounds were inoculated with 50 μl of 2 × $10^3$ conidia per milliliter aqueous spore suspensions.

[w] Percentage of wounds with either external or internal decay symptoms.

[x] Lesion diameter.

[y] Treatment means were separated using Fisher's protected LSD at $P = 0.05$. Means in each column followed by letters in common are not significantly different.

[z] No lesions observed (—).

the total spore load so that those species that occur infrequently or in low numbers were below the threshold of detection. Furthermore, although only a limited number of isolates were assayed, *P. solitum* recovered from fruit were found to be insensitive to benzimidazoles (18) and resistance to benomyl is common in the Mid-Columbia region (25). Therefore, TBZ probably did not reduce populations of many *Penicillium* spp. as much as did NaOCl and SOPP.

The concentration of several *Penicillium* spp. that were recovered from both dump tank water and drench solutions was high enough to cause potentially significant levels of decay. Although the relationships between inoculum concentration of *P. expansum* and incidence of decay of apples (2) and d'Anjou pears (24) has been established, similar relationships have not been determined for other *Penicillium* spp. On newly harvested McIntosh apples, disease incidence due to infection by *P. expansum* was asymptotic with $2.0 \times 10^3$ conidia per milliter resulting in about 50% infection and $1.5 \times 10^4$ conidia per milliter resulting in 90% infection (2). Disease incidence in d'Anjou pears that were inoculated with conidia of *P. expansum* also showed a dose/response curve that had an asymptote at about $1.5 \times 10^3$ conidia per milliter (24).

The walls and floors of wooden field bins may act as reservoirs of propagules for a number of species of fungal pathogens of pome fruit, including several *Penicillium* spp. Although the amount of inoculum contributed by field bins to the spore load in drench solutions and dump tank water is unknown, bins constitute a potentially significant source of inoculum.

Compared with that found in dump tank water and especially drench solutions, the diversity of species found on fruit was relatively low. The majority of fruit were infected with *P. expansum*, followed in order of decreasing frequency by *P. solitum*, *P. commune*, and *P. aurantiogriseum*. This level of diversity was considerably less than that occurring on pears and apples in Poland (3), but is similar to that found on apples and pears in Israel (1). Additionally, *P. verrucosum*, which is reported to be a major component of decay in New South Wales, Australia (16) was not recovered from fruit in the Mid-Columbia region and was uncommon in water systems. In comparison, *P. solitum* alone was reported to occur in more than half of the blue-mold diseased fruit in Victoria, Australia (12). In 1990–91, fruit collected from Mid-Columbia–area packinghouses were selected for typical blue-mold symptoms, and *Penicillium* spp. other than *P. expansum* were recovered infrequently. In 1991–92, fruit with lesions less typical of *P. expansum*, but similar to those of bull's-eye, Alternaria, or Cladosporium rots, also were selected. Upon isolation, a relatively high percentage of lesions yielded *Penicillium* spp. other than *P. expansum*, particularly *P. solitum*. Thus, the perceived frequency of distribution of *Penicillium* spp. can be significantly altered by sampling technique, and care must be taken to obtain unbiased samples.

Frequency of occurrence of species recovered from pears received from markets differed little from that found in packinghouses. Fruit samples from markets were probably less biased than those from packinghouses because cooperators were asked to collect all fruit culled for decay rather than selecting a specific type of decay. However, many of the fruit selected were senescent and had been culled for that reason alone. Recovery of species such as *P. digitatum* (a pathogen of citrus), *P. chrysogenum*, and *P. roquefortii* was probably due to colonization of overmature fruit in the market since these species were not pathogenic on newly harvested fruit.

The differences observed between the relative frequency and abundance of *P. solitum* found in water systems versus those found in fruit can be explained by the high level of virulence, rate of growth in fruit, and competitive ability of *P. expansum* over that of other *Penicillium* spp. *Penicillium expansum* successfully competed for wound sites over both *P. solitum* and *P. commune* in challenge inoculations made soon after initial treatments. Furthermore, once *P. expansum* became established in wounds on pears, it grew at a considerably faster rate than either *P. solitum* or *P. commune*, or any of the other species tested for pathogenicity, regardless of temperature. This would allow *P. expansum* to overwhelm any species that did not exclude it from establishment in the wound site.

The ability of *P. expansum* to become established in wounds was markedly diminished with time after initial treatment when inoculated alone or following initial treatment with *P. commune*. In uninoculated wounds this response appears to be evidence of a healing or other resistance response in wounded fruit. Evidence of wound healing has been observed in apple fruit as thickening of cell walls surrounding wound sites (14). The decrease in establishment of *P. expansum* following 7 and 28 day challenges after initial treatment with *P. commune*, however, demonstrates the inability of *P. expansum* to compete for wound sites possessed by *P. commune*.

In contrast, the establishment of *P. expansum* in older wounds appeared to be enhanced by initial treatment with *P. solitum* when fruit was stored at regular cold storage temperatures (−1 C) after the challenge inoculation. At cold storage temperatures it appears that *P. solitum* offered no competitive resistance to *P. expansum*. Furthermore, infection of wounds by *P. solitum* may afford an opening to subsequent infection by *P. expansum* through whatever barriers the fruit are capable of constructing as a wound healing response. Based on these results, *P. solitum* may have an additional role in the overall decay picture, predisposing otherwise resistant wounds to infection by *P. expansum*, and should not be viewed simply as a weak pathogen.

The number of *Penicillium* spp. that were pathogenic on newly harvested d'Anjou pear fruit was considerably less than previously stated. Only seven (*P. aurantiogriseum, P. commune, P. crustosum, P. expansum, P. griseofulvum, P. solitum,* and *P. verrucosum*) of the 12 *Penicillium* spp. tested were pathogenic on newly harvested pears. This discrepancy may be due to several factors. First is the distinct possibility that the species reported in the literature were misidentified. The zymogram system that we employed effectively removed subjectivity from species identification. Second, we tested only a limited number of isolates of each species, some of which may have been in culture for a considerable length of time and may have lost their virulence. Third, it is unclear what the condition of the fruit was at the time of the original description in previous reports and the condition of the fruit when, or if, Koch's postulates were fulfilled. As we have demonstrated, a number of *Penicillium* spp. were recovered from fruit collected in both markets and packinghouses that have not proven to be pathogenic on sound fruit (e.g., *P. roquefortii, P. digitatum,* and *P. chrysogenum*). Most *Penicillium* spp. are ubiquitous soil inhabitants that survive saprophytically on organic detritus (7,17). The demarcation between living, responding fruit and essentially dead fruit is not sharp, but instead may be seen as a continuum. Damaged and dead tissue is common in wounds and on stems and provides sites for saprophytic decay organisms to become established. It appears likely that many of the *Penicillium* spp. reported to occur on pome fruit were recovered from senescent or dead fruit.

In this study we have shown that propagules of a number of *Penicillium* spp. other than *P. expansum*, especially *P. solitum*, were present in relatively large numbers in postharvest fruit handling and treatment solutions in the Mid-Columbia region. Drench solutions were particularly rich in species, which survived immersion in TBZ. Infection by *Penicillium* spp. other than *P. expansum* may be more important than previously thought. Losses caused by these species may be direct or, as in the case of *P. solitum*, a species may be a predisposing agent that allows the entry of *P. expansum*, a far more destructive decay, into wounds. Initial infection by *P. solitum*, and possibly other of the less virulent *Penicillium* spp., served to allow entry of *P. expansum* into wounds otherwise protected by a wound-healing or other host-resistance response. Further research is needed to elucidate the nature of these responses. A systematic survey to quantify direct losses due to each species also is needed. The actual number of species that are capable of causing decay on sound, preclimacteric pear fruit appears relatively small compared to the number presented in the literature. However, *Penicillium* spp.

other than *P. expansum*, especially *P. solitum*, need to be taken into consideration when developing decay management strategies.

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