Interactions Among Fungi Causing Postharvest Decay of Pear

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ABSTRACT

Sugar, D., and Powers, K. 1986. Interactions among fungi causing postharvest decay of pear. Plant Disease 70: 1132-1134.

Phialophora malorum and *Cladosporium herbarum* cause decay lesions on pears that are indistinguishable without isolating the causal fungus. Only *P. malorum* was isolated from lesions on benomyl-treated fruit sampled from a commercial packinghouse, whereas only *C. herbarum* was found in lesions on untreated fruit. When inoculated simultaneously into pear wounds, both fungi were recovered from decay lesions after 4 mo. When one fungus was inoculated before the other, the first inoculated was more frequently isolated in pure culture from lesions. Benomyl selectively controlled infection by *C. herbarum* without inhibiting infection by *P. malorum*. Wounds inoculated with *Penicillium expansum* and either *Phialophora malorum* or *C. herbarum* developed lesions from which only *P. expansum* was recovered, but sterile filtrate of *Penicillium expansum* did not inhibit infection by *Phialophora malorum* or *C. herbarum*.

Side rot of pear (*Pyrus communis* L.) caused by *Phialophora malorum* (Kidd & Beaum.) McColloch is a major problem in the southern Oregon fruit industry, particularly in the cultivar Bosc (2). Cladosporium fruit rot, caused by *Cladosporium herbarum* Link ex Fr., cannot be distinguished from side rot by visual examination. Isolation is required for identification of the causal agent (1,3,6), which is particularly important because *C. herbarum* is sensitive to benzimidazole fungicides and *P. malorum* is not (2,6). The most common postharvest diseases of pear, blue mold (*Penicillium expansum* Link ex Thom.) and gray mold (*Botrytis cinerea* Pers. ex Fr.), are controlled by applying benomyl in the packinghouse (1,3). Spores of decay fungi are frequently found in water used in fruit handling (1,4).

Pears in southern Oregon are commonly harvested into field bins (about 454 kg/bin) and cooled to −1 C (5). To minimize handling injury to the fruit, bins are immersed in a solution containing one of several flotation salts, plus sodium ortho phenylphenate (SOPP) to reduce fungal spore populations (4). After 1–2 min of flotation, fruit are lifted from the solution on elevating belts, rinsed in fresh water, and treated with benomyl. After sorting, grading, and sizing, fruit to be marketed fresh are individually wrapped in paper and packed in polyethylene-lined fiberboard boxes (20 kg/box). Packed boxes are stored in air at −1 C.

This study was conducted to determine the identity of fungi causing side-rot-like lesions on Bosc pears at various stages in postharvest fruit handling and to account for patterns of species incidence on the basis of interactions among fungi and selective fungicides.

MATERIALS AND METHODS

Packinghouse studies. Bosc pears from an orchard with a recent history of side rot problems were sampled 7 days after harvest at three points in the handling process: 1) from field bins following cooling, 2) from elevating belts following flotation in sodium silicate + SOPP and fresh-water rinsing, and 3) as final packed boxes after treatment with benomyl at 300 ppm a.i. as a line spray. The immersion tank contained 0.45% SOPP and enough sodium silicate to raise the specific gravity of the solution to 1.05. One box of fruit (100 fruits per box) was sampled from each of 10 randomly selected field bins per treatment. All sample boxes were stored in a commercial air storage at −1 C. After 6 mo, numbers of fruit per box showing decay lesions were counted. Tissue from lesion margins of fruit surface-sterilized in 0.5% NaOCl was plated on potato-dextrose agar (PDA) (Difco) and colonies identified after 2–3 wk of incubation at 20 C.

Laboratory experiments. Bosc pears surface-sterilized in 0.5% NaOCl and rinsed with tap water were wounded to a depth of 3 mm with a sterile finishing nail 3 mm in diameter. Five wounds were made per fruit on 10 fruits per treatment. Suspensions of conidia of *P. malorum* and *C. herbarum* were prepared by flooding the surface of enough 3-wk-old colonies on PDA with distilled water to give 10,000 spores per milliliter in the final dilution. Fifty microliters of *P. malorum* conidial suspension were inoculated into each pear wound, either alone or with 50 μl of conidial suspension of *C. herbarum* added immediately or after 24 hr, 1 wk, or 2 wk. Before inoculation with the second fungus, fruit were stored at −1 C. In a second series of treatments, *C. herbarum* was inoculated first into each wound, followed by *P. malorum* according to the above schedule. In addition, 10 fruits were inoculated with combined inocula of *P. malorum* and *C. herbarum*, then dipped in a solution of 300 ppm a.i. benomyl. All fruit were stored at −1 C. After 4 mo, tissue from the margin of each lesion was plated on PDA, and colonies were identified after 2–3 wk of incubation at 20 C.

An additional series of inoculations was conducted in which 50 μl of a conidial suspension of *Penicillium expansum* was introduced into wounds also inoculated with *Phialophora malorum* or *C. herbarum*. Ten fruits inoculated with each combination were also dipped in a benomyl solution as described. A sterile filtrate of *Penicillium expansum* was prepared by drawing liquid from 2-wk-old cultures growing on nutrient broth (Difco) through a 0.45-μm membrane filter (Gelman Filters, Ann Arbor, MI). Wounded fruit were inoculated with 50 μl of filtrate, alone or with conidial suspensions of *Phialophora malorum* or *C. herbarum*. Lesions were evaluated and fungi reisolated after 4 mo of storage at −1 C.

RESULTS

Packinghouse studies. Pears sampled from field bins after precooling and after flotation in sodium silicate + SOPP developed side-rot-like lesions from which only *C. herbarum* was isolated, whereas side rot lesions on benomyl-treated pears from packed boxes yielded *P. malorum* exclusively (Table 1). Incidence of decay by *C. herbarum* was reduced by flotation in sodium silicate + SOPP. Blue mold and gray mold lesions also occurred on fruit not receiving benomyl treatment but were absent from treated fruit.

Laboratory experiments. Inoculation of *P. malorum* or *C. herbarum* into pear wounds produced lesions from which pure

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Oregon Agricultural Experiment Station Technical Paper 7801.

Accepted for publication 10 April 1986.

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Table 1. Identity of fungi causing decay of Bosc pears sampled during handling and packing (after 6 mo of storage at −1 C)

Sample stage	Percent decay[z]			
	Side rot (*Phialophora malorum*)	Cladosporium rot (*Cladosporium herbarum*)	Blue mold (*Penicillium expansum*)	Gray mold (*Botrytis cinerea*)
Precooled bins (no benomyl)	0.0 a	5.6 a	0.4	1.0
Elevator from tank (no benomyl)	0.0 a	1.8 b	1.1	1.3
Packed boxes (benomyl-treated)	3.2 b	0.0 c	0.0	0.0

[z] Values represent mean of 10 boxes (100 fruits per box) per treatment, one box sampled from each of 10 field bins (about 454 kg/bin). Numbers followed by the same letter within columns are not significantly different at $P = 0.05$ according to Duncan's new multiple range test.

Table 2. Infection by *Phialophora malorum* and *Cladosporium herbarum* inoculated into wounds in Bosc pears

Inoculation[a]	Number of wounds from which indicated fungus was recovered[b]		
	P. malorum	*C. herbarum*	*P. malorum* + *C. herbarum*
P. malorum	50	0	0
P. malorum + *C. herbarum*	3	0	47
P. malorum, then *C. herbarum* after 24 hr	10	0	40
P. malorum, then *C. herbarum* after 1 wk	48	0	2
P. malorum, then *C. herbarum* after 2 wk	48	0	2
C. herbarum	0	50	0
C. herbarum, then *P. malorum* after 24 hr	0	39	11
C. herbarum, then *P. malorum* after 1 wk	0	45	5
C. herbarum, then *P. malorum* after 2 wk	0	45	5
C. herbarum + *P. malorum* + benomyl (300 ppm a.i.)	50	0	0

[a] Fifty microliters of inoculum of each fungus at 10,000 conidia per milliliter inoculated into artificial wounds 3 mm deep × 3 mm diameter in surface-sterilized fruit.
[b] Fifty wounds were inoculated for each inoculum source. Fungi isolated from lesion margins after 4 mo storage at −1 C. Colonies identified after 2–3 wk at 20 C.

Table 3. Infection of wounds in Bosc pears by *Phialophora malorum* or *Cladosporium herbarum* when combined with *Penicillium expansum*, sterile filtrate of *P. expansum* cultures, or benomyl[a]

Inoculation[b]	Number of wounds from which indicated fungus was recovered[c]		
	P. expansum	*P. malorum*	*C. herbarum*
P. malorum	0	50	0
P. expansum	50	0	0
P. malorum + *P. expansum*	50	0	0
P. malorum + *P. expansum* + benomyl	0	50	0
P. malorum + sterile filtrate of *P. expansum*	0	50	0
C. herbarum	0	0	50
C. herbarum + *P. expansum*	50	0	0
C. herbarum + *P. expansum* + benomyl	0	0	0
C. herbarum + sterile filtrate of *P. expansum*	0	0	50
Sterile filtrate of *P. expansum*	0	0	0

[a] Benomyl (300 ppm a.i.) applied as a fruit dip.
[b] Fifty microliters of inoculum of each fungus at 10,000 conidia per milliliter inoculated into artificial wounds 3 mm deep × 3 mm diameter in surface-sterilized fruit.
[c] Fifty wounds were inoculated for each inoculum source. Fungi isolated from lesion margins after 4 mo storage at −1 C. Colonies identified after 2–3 wk at 20 C.

cultures of each species were recovered (Table 2). *P. malorum* and *C. herbarum* were recovered from fruit simultaneously inoculated with both fungi. Delayed inoculation with the second fungus until 24 hr after initial inoculation with either *P. malorum* or *C. herbarum* increased the number of lesions from which pure cultures of the first-inoculated fungus were isolated compared with simultaneous inoculation. Delays of 1 or 2 wk resulted in most lesions yielding only the first-inoculated fungus.

Inoculation of wounds with equal numbers of conidia of *Penicillium expansum* and either *Phialophora malorum* or *C. herbarum* resulted in lesions from which only *P. expansum* was recovered (Table 3). Benomyl treatment of wounds after inoculation with *Penicillium expansum* + *Phialophora malorum* resulted in lesions yielding only *P. malorum*. Fruit inoculated with *Penicillium expansum* + *C. herbarum* followed by benomyl treatment did not decay. Sterile filtrate of *P. expansum* did not inhibit infection by either *Phialophora malorum* or *C. herbarum*. *Penicillium expansum* does not inhibit mycelial growth of either of these fungi in two-member culture (*unpublished*).

DISCUSSION

Although *Phialophora malorum* and *C. herbarum* each cause a similar-appearing decay of pears, the incidence of decay caused by each species in commercial packinghouses may be regulated by the application of a selective fungicide. The results of laboratory inoculations show that *P. malorum* and *C. herbarum* can coexist in decay lesions, but if one species is inoculated before the other, the first inoculated will dominate. Therefore, the absence of *P. malorum* from fruit not treated with benomyl suggests that *C. herbarum* may infest wounds in the orchard or during handling, before *P. malorum*, and consequently dominate the resulting lesion. Packinghouse flotation tanks may be a more important source of inoculum of *P. malorum*. In benomyl-treated fruit, competition from *C. herbarum* should be eliminated, so *P. malorum* may colonize the wounds.

Because colony growth and decay of pears by *Penicillium expansum* is more rapid than by *Phialophora malorum* or *C. herbarum* (6), exclusive recovery of *Penicillium expansum* from lesions inoculated with combined inocula was apparently due to more aggressive colonization. The inability of sterile filtrate of *P. expansum* cultures to inhibit infection by *Phialophora malorum* or *C. herbarum* further suggests that this effect results from more aggressive colonization rather than antibiosis.

ACKNOWLEDGMENTS

Research supported in part by the Winter Pear Control Committee and the Fruit Growers League of

Jackson County, Oregon.

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