Relationships Between Inoculum Concentrations of Three Decay Fungi and Pear Fruit Decay

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ABSTRACT

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The relationships between inoculum concentrations of *Botrytis cinerea, Mucor piriformis,* and *Penicillium expansum* and decay of Anjou pear fruits were nonlinear and best described by quadratic regression equations. At each inoculum concentration up to 2,000 conidia per milliliter, *P. expansum* caused more decay of wounded fruits than *B. cinerea* or *M. piriformis*. However, when fruits were inoculated by immersion of stem ends, *M. piriformis* caused the highest incidence of decay. Less decay developed in spray-rinsed fruits than in nonrinsed fruits. Decay developed when pear fruits were inoculated with *B. cinerea, M. piriformis,* or *P. expansum* at various times up to 7 days at 20 C or 24 wk at −1.1 C after harvest.

Spores of fungi causing decay of pome fruits accummulate in solutions used to clean or float fruit in packinghouses (3,4). Populations of *Penicillium expansum* Lk. ex Thom. in apple-cleaning solutions varied from 200 to 12,000/ml (4) and from 1,000 to 7,000/ml in packinghouse water tanks (2). Bertrand and Saulie-Carter (1) sampled dump-tank water in apple and pear packinghouses and reported *P. expansum* spore concentrations of less than 10 and 10–100 conidia per milliliter in 47 and 44% of the samples, respectively. In addition, populations of *Mucor piriformis* Fischer were lower than 100 propagules per milliliter in 84% of the samples. Spotts and Cervantes (10) found higher populations of *Penicillium* spp. than of *Botrytis* spp. or *M. piriformis* in apple and pear packinghouse air and dump-tank water. Populations of *Penicillium* spp. and *M. piriformis* increased as the packing season progressed.

The relationship between spore numbers and decay incidence of apple has been studied (2,4), but these relationships have not been clearly established for pear. Neck rot of Anjou pear fruit resulting from inoculation of stem ends with *P. expansum* ranged from 0 to 56% at inoculum concentrations of 1×10^3–1×10^6 conidia per milliliter (5). Most decay fungi enter fruit through wounds or at the stem (pedicel) end (6,8). Changes in susceptibility to fungal invasion of pear stems after harvest have not been studied.

The purposes of this study were to establish the relationships between inoculum concentrations of *Botrytis cinerea* Pers. ex Fr., *M. piriformis*, and *P. expansum* and decay of fruit of Anjou pear (*Pyrus communis* L.) and to determine changes in susceptibility of pear stems to invasion by decay fungi after harvest. An abstract of this work has been published (9).

MATERIALS AND METHODS

Relationships between inoculum concentration and decay of wounded fruits. All decay fungi were isolated from decayed pear fruits or from air and dump-tank water in packinghouses. Strains were evaluated in preliminary experiments for virulence in mature pear fruits by incubating fruits inoculated with mycelial plugs in a moist chamber at 20 C and measuring the diameters of decayed areas after 5 days. Because packinghouse dump

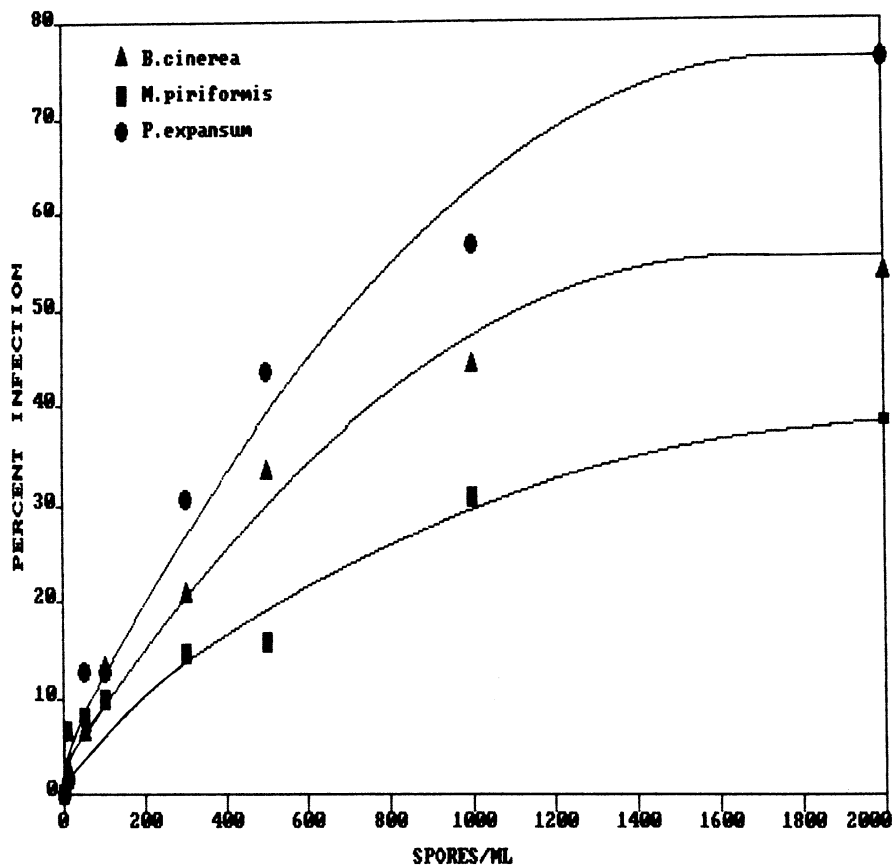

Fig. 1. Relationships between inoculum concentrations of *Botrytis cinerea, Mucor piriformis,* and *Penicillium expansum* and decay of puncture-wounded Anjou pear fruits. Fruits were rinsed after inoculation with tap water under a laboratory faucet. *B. cinerea*: $y = 3.06 + 0.06\,(x) - 0.00002\,(x^2)\ r^2 = 0.98$. *M. piriformis*: $y = 4.53 + 0.03\,(x) - 0.000008\,(x^2)\ r^2 = 0.96$. *P. expansum*: $y = 4.52 + 0.08\,(x) - 0.00002\,(x^2)\ r^2 = 0.98$.

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tanks contain a mixture of strains of varying virulence (10), two or three strains of each fungus that varied from low to high virulence were selected for decay experiments. Fungi were grown on potato-dextrose agar (Difco) acidified with 1.5 ml of 85% lactic acid per liter. Conidia were harvested from 1- to 2-wk-old cultures with water, and suspensions were adjusted with a hemacytometer to obtain concentrations of 0, 10, 50, 100, 300, 500, 1,000, and 2,000 conidia per milliliter.

Mature Anjou pear fruits were harvested and surface-sterilized with 0.525% sodium hypochlorite, rinsed with water, and air-dried. Fruits were puncture-wounded (wounds 6 mm in diameter and 3 mm deep) on four locations per fruit. Spore suspension temperatures were equilibrated to 10 C, and 10–12 wounded fruits were immersed for 5 min in each concentration, removed, and rinsed with tap water under either a laboratory faucet or a series of nozzles on a packingline. Fruits were faucet-rinsed for 5 sec with 600 ml of water. The spray rinse, involving five flat fan-pattern nozzles, each delivering 3 L/min, was applied to fruits moving at 3.8 m/min on 0.61-m-wide rotating brushes. A separate lot of fruits treated in 1,000 conidia per milliliter was not rinsed.

Treated fruits were placed in polyethylene-lined boxes for incubation. The percentage of wounds with decay was evaluated after 5–8 days at 20 C. All experiments were repeated. Relationships between spore concentrations and decay were examined with regression analysis (NWA Statpak, Northwest Analytical, Portland, OR).

Relationships between inoculum concentrations and stem-end decay of nonpunctured fruits. Mature Anjou pear fruits were surface-sterilized as described earlier and rinsed, then 15–20 fruits were submerged in 0–2,000 conidia per milliliter of each fungus for 5 min. Fruits were rinsed with tap water under a laboratory faucet, then stored in polyethylene-lined boxes at −1.1 C and evaluated monthly for decay. After 3–6 mo of storage, fruits were ripened at 20 C for 7 days and final decay evaluations were done. Incidence of decay from all evaluations of each fungus was combined to give total incidence of wounds with decay for each fungus at each concentration.

Changes in susceptibility to stem-end decay after harvest. To determine the time that stem ends remain susceptible to fungal invasion after harvest, Anjou pear fruits were harvested, surface-sterilized, and rinsed with sterile distilled water. Fruits were stored in polyethylene-lined boxes at 20 C for 1, 2, 5, and 7 days or at −1.1 C for 2, 4, 6, 8, and 24 wk before inoculation. At each time, the stem ends of 10 fruits were dipped in sterile distilled water or 5×10^4 conidia of *B. cinerea, M. piriformis,* or *P. expansum* per milliliter. Stem ends were air-dried and fruits stored at −1.1 C. Seven months after harvest, the fruits were ripened for 10 days at 20 C and incidence of fruits with decay was evaluated.

RESULTS

The relationships between inoculum concentrations of *B. cinerea, M. piriformis,* and *P. expansum* and decay were described with quadratic regression equations. Levels of decay caused by *P. expansum* after stem-end inoculation were described by simple linear regression. As inoculum concentration increased, the ratio between infection and inoculum concentration decreased, except with decay caused by *B. cinerea* after a spray rinse and by *P. expansum* after stem-end inoculation. Generally, slopes of the regression curves were greatest when inoculum concentrations were less than 1,000 conidia per milliliter (Figs. 1–3). At each inoculum concentration, *P. expansum* caused more decay of wounded fruits than *B. cinerea* or *M. piriformis* (Figs. 1 and 2). However, *M. piriformis* caused the greatest amount of stem-end decay at each inoculum concentration (Fig. 3). Typically, less than 3% of the wounds in uninoculated control fruits were infected because of contamination.

The method of rinsing affected the amount of fruit decay caused by all three fungi (Table 1). A faucet rinse significantly ($P = 0.05$) reduced decay caused by *P. expansum* compared with no rinse. A spray rinse significantly ($P = 0.05$)

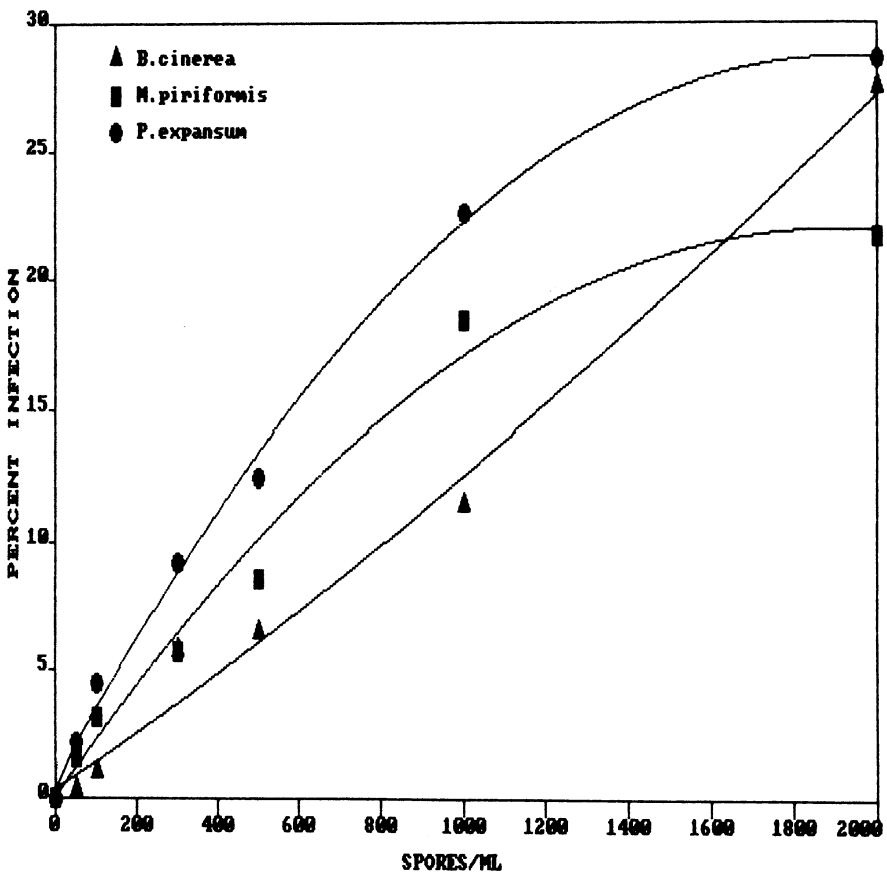

Fig. 2. Relationships between inoculum concentrations of *Botrytis cinerea, Mucor piriformis,* and *Penicillium expansum* and decay of puncture-wounded Anjou pear fruits given a postinoculation tap water rinse with line-spray nozzles. *B. cinerea*: $y = 0.35 + 0.01\,(x) + 0.000001\,(x^2)$ $r^2 = 0.99$. *M. piriformis*: $y = 0.08 + 0.02\,(x) - 0.000006\,(x^2)$ $r^2 = 0.99$. *P. expansum*: $y = 0.72 + 0.03\,(x) - 0.000007\,(x^2)$ $r^2 = 0.99$.

Table 1. Effect of water rinsing on decay of puncture-wounded Anjou pear fruits by three fungi

Method of rinse[y]	Percentage of wounds decayed by[z]		
	Botrytis cinerea	*Mucor piriformis*	*Penicillium expansum*
No rinse	79 a	42 a	81 a
Faucet	55 ab	32 ab	57 b
Line spray	12 b	20 b	34 c

[y] Tap water used for all rinses.
[z] Fruits inoculated before rinse by immersion for 5 min in water at 10 C containing 1×10^3 conidia per milliliter of each fungus. Two strains of each fungus used and experiment repeated once.

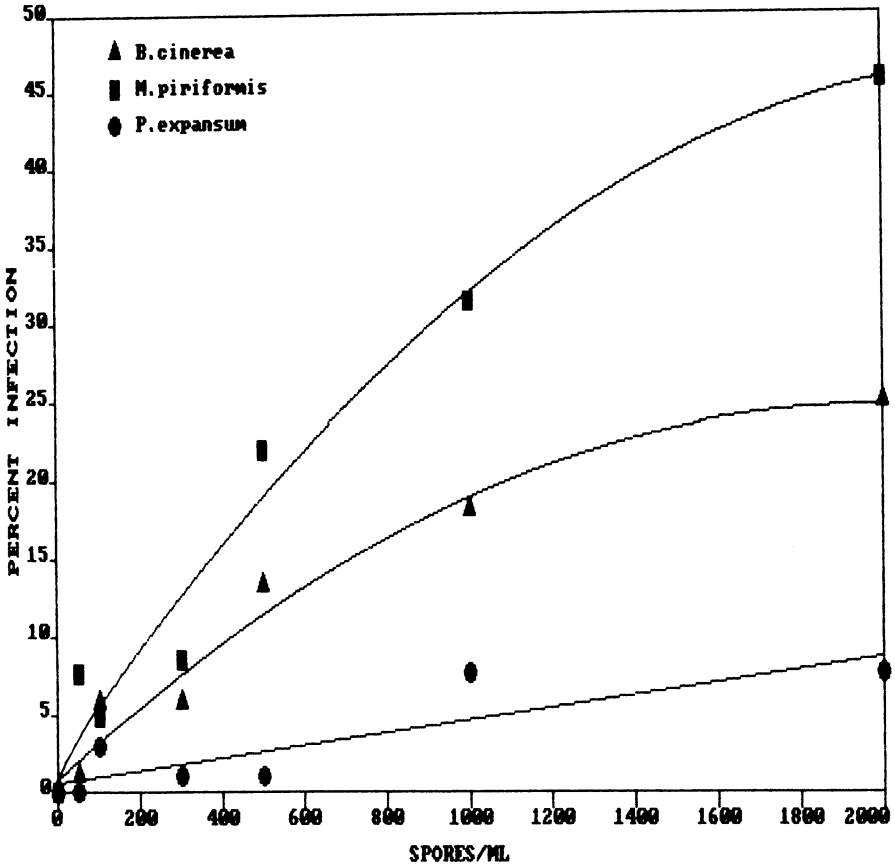

Fig. 3. Relationships between inoculum concentration of *Botrytis cinerea, Mucor piriformis,* and *Penicillium expansum* and stem-end decay of nonpunctured Anjou pear fruits. *B. cinerea*: $y = 0.81 + 0.02\,(x) - 0.000006\,(x^2)$ $r^2 = 0.97$. *M. piriformis*: $y = 1.8 + 0.04\,(x) - 0.000008\,(x^2)$ $r^2 = 0.97$. *P. expansum*: $y = 0.60 + 0.004\,(x)$ $r^2 = 0.74$.

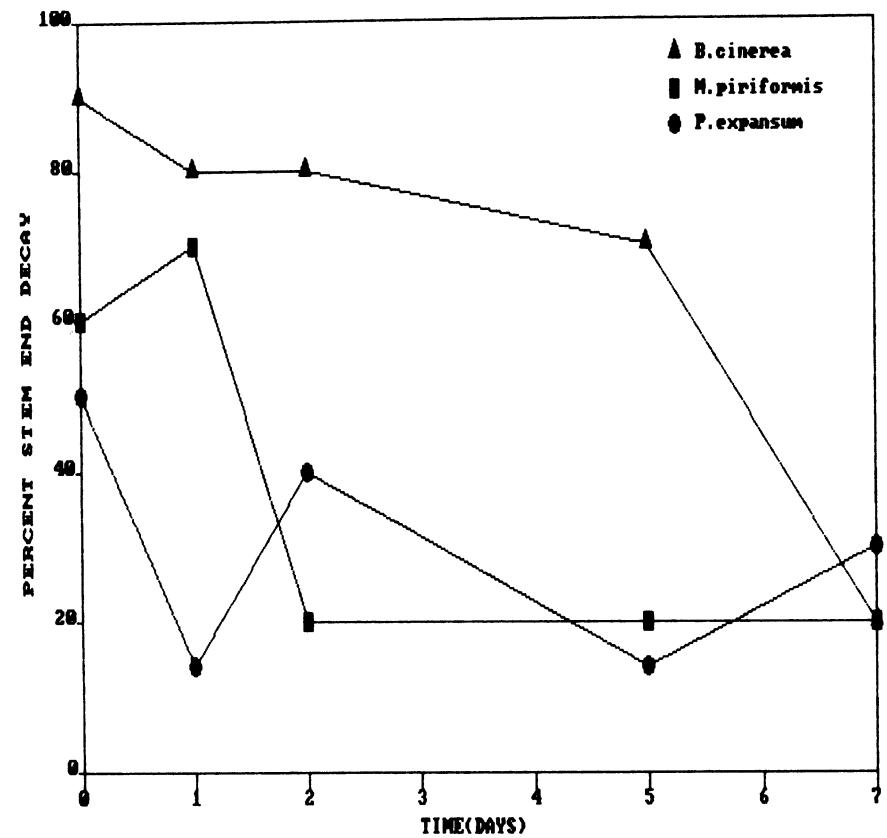

Fig. 4. Effect of time at 20 C between harvest and inoculation of stem ends with *Botrytis cinerea, Mucor piriformis,* and *Penicillium expansum* and decay of Anjou pear fruits. Fruit stems were inoculated with 5×10^4 conidia per milliliter.

reduced decay caused by all three fungi compared with no rinse and was more effective than the faucet rinse for control of *P. expansum*.

Decay resulting from inoculation of stem ends occurred in fruit kept at 20 C up to 7 days before inoculation (Fig. 4). Less decay developed from *B. cinerea* and *M. piriformis* in fruits held for 7 and 2 days, respectively, than in fruits inoculated within 1 day of harvest, but no pattern was obvious for *P. expansum* (Fig. 4). When pear fruits were stored at −1.1 C and inoculated by stem dip at various times up to 24 wk after harvest, decay developed at all inoculation times (Table 2). No decay from *B. cinerea* or *P. expansum* developed in control fruit, and *M. piriformis* caused 13% stem decay of control fruit. Although decay from *B. cinerea* and *M. piriformis* developed in puncture-wounded fruits within 4 wk and decay from *P. expansum* developed within 8 wk at −1.1 C, 16–19 wk were required before decay developed in fruits when stem ends were dipped in *B. cinerea* and *M. piriformis* and incubated at −1.1 C. Decay caused by *P. expansum* from stem-end inoculation occurred only after fruits were ripened 10 days at 20 C after the 7-mo storage period.

DISCUSSION

Populations of *Botrytis* spp., *Penicillium* spp., and *M. piriformis* in packinghouse dump tanks in the Mid-Columbia region of Oregon and Washington have been described quantitatively (10), and concentrations used in this study were similar to those commonly found in commercial packinghouses. The relationships between inoculum concentration and decay were complex, and simple threshold spore concentrations could not be established for industry use. However, several important characteristics of the disease incidence/inoculum dose curves were apparent. First, all curves pass through the origin and have the greatest slopes at inoculum concentrations under about 1,000 conidia per milliliter. Thus, it is important that packinghouse managers reduce spore concentrations in dump tanks and flumes to the lowest possible level, because any viable inoculum will lead to decay when wounds are present. Second, the curves show an asymptote at about 1,500 conidia per milliliter. This suggests that potential infection courts have become saturated with inoculum at this conidial concentration and represents one of the most common relationships between disease incidence and inoculum dose (11). The saturation concentrations for *B. cinerea* after a spray rinse or for *P. expansum* after stem-end inoculation were not reached in this study. Packinghouses are advised to minimize dump-tank spore concentrations with chlorine or sodium ortho phenylphenate (1).

A spray rinse reduced decay in this

Table 2. Decay of Anjou pear fruits from stem-end inoculation with three fungi after harvest

Time from harvest to inoculation[a] (wk)	Percentage of fruit decayed by[b]		
	Botrytis cinerea	*Mucor piriformis*	*Penicillium expansum*
0	90	60	50
1	70	60	50
2	80	40	26
4	80	70	12
6	90	40	37
8	70	60	12
24	14	42	37

[a] Fruits stored at −1.1 C after harvest (before and after inoculation). Fruits inoculated by dipping stem end into 5×10^4 conidia per milliliter of each fungus.
[b] Each value represents 10 fruits. Seven months after harvest, fruits were ripened at 20 C for 10 days and decay was evaluated.

study and also in a previous study (3). This effect probably is related to physical removal of spores from infection courts. A faucet rinse, which appeared less thorough than the spray rinse involving rotation of fruits on brushes, had an intermediate effect, resulting in incidence of decay between those of the line spray and the nonrinsed treatment.

Fruit decay originating from fungal invasion through the stem end is a serious problem (6,7). If fruits were held for 2 days at 20 C, stem end decay caused by *M. piriformis* was reduced, but decay caused by *B. cinerea* or *P. expansum* was not controlled. Thus this treatment does not appear to be an acceptable decay-control strategy. Stems of fruit held at −1.1 C remained susceptible to invasion and subsequent decay even after 24 wk. Because commercial storage facilities often keep pear fruit in bins at −1.1 C for more than 6 mo before flotation through contaminated dump tanks in the packing process, care must be taken to reduce inoculum concentration during these late-season operations. The incubation period for fruit decay from stem-end infections is 16–19 wk, and fruit lots with high potential for decay could be marketed before this time to reduce losses.

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