Dispersal of Inoculum of *Phialophora malorum* in Pear Orchards and Inoculum Redistribution in Pear Immersion Tanks

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ABSTRACT

Sugar, D., and Spotts, R. A. 1993. Dispersal of inoculum of *Phialophora malorum* in pear orchards and inoculum redistribution in pear immersion tanks. Plant Dis. 77:47-49.

Phialophora malorum was recovered from orchard air during fruit harvest and from surfaces of harvested fruit in orchard bins but not from orchard air or fruit surfaces during the growing season. Propagules of *P. malorum* were recovered from immersion dump tank solutions in a commercial packinghouse but not from packinghouse air. Redistribution of inoculum from infested to previously uninfested fruit was demonstrated in a research-scale immersion tank system. Side rot did not develop in surface-sterilized fruit sampled during commercial packing operations, but it did occur in samples that were not surface-sterilized. Results support the hypothesis that soilborne or deposited airborne spores may be brought into packinghouses on fruit surfaces or harvest bins and be redistributed to uninfested fruit in immersion tank solutions.

Additional keywords: postharvest decay, *Pyrus communis*

Side rot of pear, caused by *Phialophora malorum* (M.N. Kidd & A. Beaumont) McColloch, is an important disease of stored pears in the Rogue River Valley of southern Oregon (3,4). *P. malorum* is a resident in orchard soil (22), which may be an important inoculum source for postharvest fruit decay. The means by which propagules of *P. malorum* contact fruit prior to infection is unknown. McColloch (13) stated in 1944 that apples become infected by *P. malorum* while on the tree but presented no data to support this claim.

Michailides and Spotts (14) found *Mucor piriformis* E. Fisch., another postharvest pathogen of pear, in orchard soil and debris, in decaying fallen fruit on the orchard floor, in soil adhering to harvest bins, and in packinghouse immersion dump tanks. *M. piriformis* was not recovered from orchard air or fruit sampled during harvest. They concluded that *M. piriformis* contacts pear fruit in immersion tank solutions infested by spores brought from the orchard in soil and debris associated with harvest bins. Several postharvest pathogens of pear and apple have been found contaminating immersion tank solutions (5–8,11,18). Other postharvest pathogens, namely, *Botrytis cinerea* Pers.: Fr., *Penicillium expansum* Link, and *Cladosporium herbarum* (Pers.:Fr.) Link have been associated with decaying plant debris in soil (1,2,10,15) and were found in orchard air and in washings of pear fruit while on the tree (14). *Alternaria alternata* (Fr.:Fr.) Keissl. was also recovered from orchard air (14).

The objective of this study was to determine how and when inoculum of *P. malorum* is dispersed from orchard soil to pear fruit. Since immersion dump tanks are known to harbor spores of pathogenic fungi (5,7,8,11,16,18), additional experiments were designed to evaluate the potential for exchange of spores of *P. malorum* between fruit surfaces and the dump tank solution.

MATERIALS AND METHODS

Orchard studies. To determine if pears become infested while on the tree or during harvest operations, studies were conducted in a commercial orchard near Medford, Oregon, with a history of side rot incidence. Orchard air was sampled at 2-wk intervals for 18 mo beginning in July, 1987. Ten petri dishes of potato-dextrose agar (PDA) were placed on the soil surface at random sites throughout a 0.5-ha test area, and covers were removed for 5 min. The petri dishes were then brought to the laboratory and evaluated for development of *P. malorum* colonies after 2- to 4-wk incubation at 20 C. To determine when fruit become infested, 20 fruit on each of five randomly chosen trees were washed every 2 wk during 1987 and 1988 from late June through harvest. Approximately 2 ml of distilled water was sprayed onto the surface of each fruit with a hand-operated atomizer, and runoff water was collected in a sterile plastic beaker. Runoff water from all 20 fruit on an individual tree was pooled and centrifuged 10 min at 2,000 rpm to concentrate spores. A 0.5-ml aliquot was drawn from the bottom of each centrifuge tube and spread onto a semiselective medium containing benomyl, 2,6-dichloro-4-nitroaniline, streptomycin sulfate, and rose bengal (22). Washes were made of 20 fruit from each of five randomly chosen standard harvest bins in the orchard test area at harvest each year. Bins contained approximately 454 kg of fruit.

In a second study to determine the time of fruit infestation, 10 fruit on each of five randomly selected trees were covered with paper bags, which were closed around the fruit spur with plastic tape to prevent deposition of spores. Fruit were bagged every 2 wk beginning in late July of 1987 and 1988 and remained covered through harvest. Fruit were harvested while in bags and stored 1 wk at 0 C. Fruit from each bagging date from each replicate tree were then individually sprayed with benomyl (300 mg a.i./L) to reduce competition from fungi other than *P. malorum*, which is not sensitive to benomyl (4,20). Fruit were stored in polyethylene-lined boxes for 5 mo at 0 C, at which time decay was evaluated and causal fungi identified. Ten nonbagged fruit were harvested from each tree as controls.

To further evaluate whether or not latent infections were present before harvest, fruit from the test orchard were sampled during commercial packing. Ten 100-fruit replicate samples were collected at 20-min intervals from packing lines after fruit had passed through the immersion dump tank. The fruit was then surface-sterilized by 5 min immersion in water containing 0.5% NaOCl, and stored for 5 mo at −1 C in polyethylene-lined cardboard cartons. Side rot incidence in the treated fruit was compared to that in 10 commercially packed boxes randomly selected during the same time period that had not received NaOCl dips. Identity of causal fungi was confirmed by plating diseased tissue from lesion margins on PDA in petri dishes. *P. malorum* was identified by colony morphology and color and by microscopic examination of the shape and size of conidiogenous cells and conidia (13). Treatment means were compared by Student's *t* test for unpaired data.

Packinghouse studies. To determine if pear fruit become infested with *P. malorum* during the packing process, samples of dump tank solutions and packinghouse air were taken before and after pear dumping, and the number of propagules of *P. malorum* was determined. Samples were taken several times during

Accepted for publication 17 September 1992.

commercial packing of Bosc pears in 1985 and 1991. In most pear packinghouses in southern Oregon, pears in wooden harvest bins (approximately 454 kg/bin) are immersed in water containing a flotation salt and sodium *o*-phenyl phenate (SOPP) as a disinfestant (9,12, 17,21). In the packinghouse, immersion tanks were emptied, cleaned, and refilled with either sodium carbonate (1985) or sodium lignin sulfonate (1991) to specific gravity 1.04 and 0.3% SOPP before packing on the first and fourth day of each week. Fresh tank solutions were sampled before pear dumping on the first day of each week for 3 wk, and samples were taken 30–90 min after pear dumping had begun on each of the first 3 days of each week. Five replicate 1.0-ml aliquots of tank solution were withdrawn by pipette and immediately diluted in 99 ml of distilled water. A 0.5-ml aliquot of the dilution was spread on each of five petri dishes of PDA, and colonies that developed were counted after 2- to 4-wk incubation at 20 C. Packinghouse air was sampled on each date by placing four petri dishes of PDA on the surfaces of packinghouse equipment surrounding the immersion tank and removing the covers for 5 min. The petri dishes were then brought to the laboratory and evaluated for development of *P. malorum* colonies after 2- to 4-wk incubation at 20 C.

Table 1. Recovery of *Phialophora malorum* from immersion dump tank solutions in a commercial pear packinghouse in Medford, Oregon

Sample date[a]	After dumping[b] (spores/ml ± SD)
1985	
21 Oct.	48 ± 34
22 Oct.	320 ± 94
23 Oct.	112 ± 52
28 Oct.	104 ± 83
29 Oct.	0
30 Oct.	230 ± 76
4 Nov.	352 ± 77
5 Nov.	120 ± 40
6 Nov.	216 ± 36
1991	
7 Oct.	72 ± 60
8 Oct.	32 ± 34
9 Oct.	0
14 Oct.	32 ± 34
15 Oct.	48 ± 18
16 Oct.	72 ± 44
28 Oct.	16 ± 22
29 Oct.	0
30 Oct.	0

[a] On the first packing day of each week, tanks were drained, cleaned, and filled with fresh water, sodium *o*-phenyl phenate (0.3%), and either sodium carbonate (1985) or sodium lignin sulfonate (1991) to a specific gravity of 1.04. Samples taken before dumping of pears contained no spores.

[b] Samples taken from dump tank solution 30–90 min after dumping of pears began each day.

Inoculum redistribution in immersion tanks. To measure the potential for transfer of spores from fruit to the tank solution, surfaces of 10 Bosc pears were sprayed, using a hand-operated atomizer, with water containing spores of *P. malorum*. The spore suspension was prepared by washing 2- to 4-wk-old colonies growing on PDA with distilled water; then spore concentration was determined with a hemacytometer and adjusted to 1×10^4 conidia/ml by dilution with distilled water. After air-drying on a laminar-flow sterile bench, five of the fruit were peeled, and peels were weighed and blended in 20 ml of distilled water for 5 min in a Waring Blendor at high speed. Five 0.5-ml aliquots of the blended suspension were spread on PDA in petri dishes, and colonies of *P. malorum* were counted after 2- to 4-wk incubation at 20 C. The remaining five fruit were individually immersed for 2 min in a circulating water bath (VWR model 1165) containing 3 L of tap water at 10 C; then peels were air-dried, removed, blended, and plated as above. The water was changed, and the tank was cleaned with 95% ethanol after each fruit immersion and sampling. Five 0.5-ml aliquots were removed from the water before and after immersion of each fruit and spread on PDA as described above.

A second experiment was designed to test the potential for previously uninfested fruit to become infested following dump tank immersion. Five Bosc pears were surface-sterilized for 5 min in 0.5% NaOCl, rinsed in tap water, air-dried, and then individually immersed 2 min in a circulating water bath containing 1×10^4 spores of *P. malorum* per milliliter. The water was changed, and the tank was cleaned after immersion and sampling of each fruit. The number of spores that were carried from the bath on fruit surfaces was measured by peeling, blending, and culturing on PDA as above. Both experiments were done twice.

RESULTS

Orchard studies. *P. malorum* was not recovered from orchard air during either the 1987 or 1988 growing seasons, but was detected in air samples collected on a single date (1 Sept. 1987) during commercial fruit harvest. On that date, a total of seven colonies of *P. malorum* developed on five of the 10 sample plates. *P. malorum* was not recovered from fruit surfaces during either growing season, but it was detected on fruit in seven of 10 harvest bins sampled in the orchard in 1987. No side rot developed in fruit covered with paper bags for any portion of the growing season nor in fruit from the same trees that were not bagged. No side rot developed during storage in fruit immersed in NaOCl solution after passage through the commercial dump tank, whereas side rot occurred in an average of 1.1% of the commercially packed fruit that was not similarly disinfested.

Packinghouse studies. *P. malorum* was not recovered from packinghouse air on any sampling date, nor was it recovered from immersion tank solutions before dumping of pears. However, *P. malorum* was recovered from tank solutions after bins of pears had been immersed on most sampling dates (Table 1). The mean concentration of propagules of *P. malorum* in the tank solutions ranged from none to 352 per milliliter.

Inoculum redistribution in immersion tanks. Immersion of artificially infested pears for 2 min in a circulating water bath resulted in an average of 59% of the spores of *P. malorum* being transferred from the fruit surface to the tank water (Table 2). Surface-sterilized fruit immersed for 2 min in the circulating bath containing 10^4 spores of *P. malorum* per milliliter carried an average of 764.9 spores per gram of peel after immersion.

DISCUSSION

Frequent recovery of *P. malorum* from packinghouse tank solutions during pear dumping but not from packinghouse air nor from fresh tank solutions indicated that propagules of *P. malorum* were car-

Table 2. Transfer of spores of *Phialophora malorum* between surfaces of Bosc pears and a circulating water bath as a simulated packinghouse immersion tank

Sample	Fruit infested before immersion in water bath[a,c]	Sterile fruit immersed in infested water bath[b,c]
Fruit surface, cfu/g peel		
Before immersion	1,806.1	0.0
After immersion	739.1	764.9
Water bath, cfu/ml		
Before immersion	0.0	...
After immersion	40.8	...

[a] Fruit was infested by spraying with a suspension containing 10^4 conidia of *P. malorum* per milliliter. Following immersion, peels were removed from air-dried fruit and blended in 20 ml of sterile distilled water; five 0.5-ml aliquots per fruit were plated on potato-dextrose agar.

[b] The infested tap water bath contained 10^4 conidia per milliliter. Water bath solution samples were plated without blending.

[c] Values represent means of five replicates. Means within all before versus after immersion pairs are significantly different according to Student's *t* test for unpaired data.

ried into the packinghouse on fruit and/or on harvest bins. Since *P. malorum* is known to be resident in orchard soil (22), the fungus could be carried in soil adhering to bins during harvest. Bins are moved about in the orchard by tractor-mounted forklifts, and they often become contaminated with soil (14). Recovery of *P. malorum* from fruit surfaces and orchard air during harvest in 1987 may be attributable to spores borne in dust raised during the harvest. In a related study, propagule levels of *P. malorum* in orchard soil were relatively high during the harvest period (22). Furthermore, dry, dusty conditions prevailed during the 1987 harvest. The lack of fruit contamination prior to harvest, together with the development of side rot in commercially packed but not in surface-sterilized fruit, indicates that latent infection of fruit while on the tree is unlikely. Washings of pear fruit and bagged-fruit experiments in this study also provided evidence that infestation of fruit on the tree does not commonly occur, since no side rot developed in either treatments or controls in either year of study. However, fruit may become infested on the tree during harvest operations, since we recovered *P. malorum* from harvested pears in bins while in the orchard. Fruit washed or bagged while on the tree may have escaped infestation by soilborne *P. malorum*, or other conditions for infection, such as injury during harvest or handling, may have been lacking.

The partial loss of spores of *P. malorum* from pear fruit surfaces during immersion in a circulating water bath, and the aquisition of spores during immersion of surface-sterile fruit in a spore-suspension bath suggest that spore transfer is likely to occur in packinghouse immersion tanks. Most spores entering an immersion tank are inactivated by contact with disinfestants present in the solution (19), but as shown in this and other studies (7,8,19,20), significant numbers of viable spores also may be present. Thus, the immersion tank may be a critical site of redistribution of inoculum of *P. malorum*; fruit may occasionally become infested while in the orchard, whereas others become infested in the immersion tank with spores transferred from orchard-infested fruit or from soil contaminating harvest bins. The amount of inoculum entering the immersion tank could potentially be reduced by avoiding contamination of harvest bins with orchard soil and washing bins and/or fruit with a nonrecirculating drench before entering the packinghouse. The viability of inoculum in the immersion tank can be minimized by selection of the most effective disinfestants (19,21) and maintaining them at appropriate concentrations.

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