Sources of Inoculum of *Phialophora malorum*, Causal Agent of Side Rot of Pear

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ABSTRACT

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Phialophora malorum was found in pear orchard soil over a 2-yr period. *P. malorum* was not a primary colonizer of fallen fruit on orchard soil, but propagule numbers increased subsequent to fruit decay by other organisms. Cankers developed when *P. malorum* was inoculated into injured bark of pear trees but not when spore suspensions were held in contact with uninjured bark. Cankers were nonperennial, and *P. malorum* was not recovered from cankered tissue after one season. The fungus was isolated in washings of bark of pear trees, but dispersal of inoculum from artificially infested bark to other areas of bark on the same trees was not observed.

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 (1,2,11). Side rot is a disease of long-term stored fruit, rarely observed before 3 mo of storage and appearing more frequently after pears have been stored 4–5 mo at −1 C (11). Although characteristics of *P. malorum* have been described in culture and from examinations of decay lesions in apples and pears (2,5–7,9,10), little is known about sources of inoculum for fruit decay. In a study of *P. malorum* as a cause of apple rot in the eastern United States, McColloch (9) stated that the fungus lives saprophytically in surface soil, on bark, and in cankerous woody tissue of apple trees, and that apples become infected while on the tree. He recovered one isolate from soil beneath a tree and another from a cankerous branch area. However, the roles of these potential inoculum sources in the disease cycle were not identified, nor were data provided to substantiate infection of fruit while on the tree.

The objective of this study was to determine sources of inoculum of *P. malorum* for postharvest pear decay. Results of related experiments indicated that *P. malorum* enters packinghouse immersion dump tank solutions on fruit surfaces or in soil carried on or in harvest bins (12). Therefore, these investigations focused on soil, tree surfaces, and tree cankers as potential orchard sources of inoculum.

MATERIALS AND METHODS

Semiselective medium. Because *P. malorum* colonies develop slowly even at optimum temperatures (6,9), a semiselective medium (SSM) was developed for enumeration of *P. malorum* populations in soil and on plant surfaces. The following medium proved successful in excluding most competitive organisms: 39 g of potato-dextrose agar (PDA) (Difco, Detroit, MI), 200 mg of benomyl (Benlate 50W, DuPont, Wilmington, DE), 99 mg of 2,6-dichloro-4-nitroaniline (Botran 75W, Upjohn, Kalamazoo, MI), 300 mg of streptomycin sulfate (Agri-Strep, Merck, Rahway, NJ), and 41.5 mg of rose bengal (J. T. Baker, Phillipsburg, NJ) (8) per liter of distilled water. All antimicrobial compounds were added to partially cooled agar after autoclaving. Although *Alternaria* spp. grew on this medium, colony expansion was restricted by the rose bengal, allowing enumeration of *P. malorum* colonies.

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Populations of *P. malorum* in orchard soil. A pear orchard that had a history of side rot problems was used as a study site. The orchard consisted primarily of the cultivar Bosc and was located near Medford, OR. Ten mature trees were randomly chosen within the block. At 2- to 4-wk intervals over a 16-mo period, approximately 50 g of soil from the top 4 cm below the duff were collected from each of four sites around the drip line of each tree. Samples from each tree were thoroughly mixed. Ten grams of soil from each tree site was added to 90 ml of distilled water in an Ehrlenmeyer flask and agitated for 20 min on a wrist-action shaker. One milliliter of soil solution was withdrawn from each flask and diluted in 99 ml of distilled water. After stirring, 10 0.5-ml aliquots of the dilution from each flask were plated on SSM. Spore washes from stock cultures of *P. malorum* also were spread on two SSM plates at each sampling date as a reference for colony development. After 6–8 wk of incubation at 20 C, colonies of *P. malorum* were identified and counted on each plate. Identification was based on colony morphology and color as well as on the shape and size of conidiogenous cells and conidia (9). Five grams of soil from each tree site also was dried overnight to constant weight in an oven at 100 C, and moisture content was determined. Results were expressed as the number of propagules per gram of dry soil. During January and February 1989, additional soil samples were collected at three of the tree sites. Samples were collected from four locations relative to the tree: adjacent to the trunk, at midcanopy (halfway from trunk to drip line), at the drip line, and in open areas halfway between tree rows. Propagules of *P. malorum* in the soil samples were enumerated using the above procedures.

The relative virulence of 45 isolates of *P. malorum* from soil was tested by inoculating artificial wounds in Bosc pears with 0.05 ml of a spore suspension containing 10^5 spores per milliliter, and by comparing lesion diameters after 3 mo of incubation at 0 C. Wounds were made by puncturing fruit to a 3-mm depth with the head of a sterile finishing nail 6 mm in diameter.

Population dynamics in artificially infested soil. Twenty-seven 3.79-L plastic nursery pots were filled with nonsterile potting mix and placed outdoors on wooden benches. An aqueous spore suspension of *P. malorum* (10^5 conidia per milliliter) was prepared from 3-wk-old colonies growing on PDA. To each of 18 pots, 100 ml of the spore suspension was added and mixed thoroughly with the potting mix. Isolates used had been recovered previously from lesions on pear fruit. The remaining nine pots served as uninoculated controls. In one half of the inoculated pots, freshly harvested, mature Bosc pear fruit were positioned on their sides half-buried in the soil. Soil in all pots was kept moist by weekly addition of approximately 100 ml of tap water. Water was added

twice weekly during dry periods. At 2- to 4-wk intervals over a 9-mo period, approximately 5 g of soil from the 2.5 cm of surface soil in each pot was collected, and populations of *P. malorum* were determined using dilution plating on SSM as described above. In pots containing pear fruit, soil was sampled within a zone approximately 2.5 cm from the fruit. As fruit in the soil began to visibly decay, isolations were made from decaying tissue to determine whether *P. malorum* was present. During this same period, isolations were made from decayed areas on five fallen fruit on and in soil at each of the 10 orchard soil sampling sites. Fruit were surface-sterilized for 5 min in 0.5% NaOCl, and decayed tissue was plated on PDA. After analysis of variance, treatment data were compared by Fisher's protected least significant difference test, using Number Cruncher Statistical System software (J. L. Hintze, Kaysville, UT).

Canker development studies. Bosc pear trees at the Southern Oregon Experiment Station were inoculated with *P. malorum* at monthly intervals over an 18-mo period beginning in July 1987. Side rot had not previously been observed in fruit from this orchard. On each of five replicate trees, one 10-mm disk of bark was removed from 3-yr-old wood and replaced either with a disk of mycelium from 2- to 4-wk-old cultures of *P. malorum* growing on PDA or with a sterile disk of PDA as a control. Inoculation sites were covered for 1 mo with Parafilm (American Can Co., Greenwich, CT). In addition, 1-cm sections of plastic tubing (4-mm internal diameter) with both ends open were placed on the bark surface of 1-, 2-, and 3-yr-old wood, perpendicular to the branch axis. Contact points between the tubes and bark surface were sealed with modeling clay. Tubes then were filled either with 0.1 ml of a spore suspension of *P. malorum* (10^5 spores per milliliter) or with sterile distilled water as a control. The aqueous suspensions were prepared from 2- to 4-wk-old colonies growing on PDA. Open ends of the tubes were covered with Parafilm. After 1 mo, tubes and clay were removed and discarded. Inoculations with spore suspensions began in December 1986 and continued monthly through January 1989.

Canker development was assessed periodically from August 1987 to July 1989. Canker length was measured between the farthest points of visibly cankered tissue along the axis of the tree branch. Isolations were made from canker margins to determine the presence of *P. malorum*. Isolations also were made at regular intervals during the spring and summer of 1988 from 10 cankers to determine the continuing presence of *P. malorum* in cankers. These isolations were made from cankers that developed after mycelial inoculations in the fall and early winter of 1987. In addition, in the orchard where populations of *P. malorum* were assayed in the soil, isolations were made from suspected cankers in trees at each soil sampling date.

Five cankers that developed from mycelial inoculation in 1987 were washed at 2- to 4-wk intervals over 14 mo, beginning in June 1988, to enumerate spore populations on the canker surface. Cankers were sprayed with 0.5 ml of distilled water from a hand-held atomizer, and runoff was collected and spread on SSM plates. Plates were incubated and evaluated as described above.

Survival of *P. malorum* on bark. Where cankers did not develop from spore suspensions held in plastic tubing on the bark of 3-yr-old wood, inoculation sites were washed by spraying with 0.5 ml of distilled water. The water was collected and plated on SSM as described above. Washes were made monthly from September to December 1988, beginning 2 wk after the final inoculation by spore suspension. Control sites were washed similarly.

RESULTS

Populations of *P. malorum* in orchard soil. *P. malorum* was recovered from orchard soil by dilution plating on SSM. Although propagule levels were highly variable, the number of propagules appeared to increase in late summer and reach relatively high levels close to the time of pear harvest in late August to early September (Fig. 1). *P. malorum* was recovered from all four locations relative to the trees. Soil populations were not high during the sampling period, ranging from 77 to 397 colony-forming units per gram of dry soil. All isolates evaluated for virulence caused lesion development on Bosc pears. Mean lesion diameters after 3 mo of incubation ranged from 11.6 to 14.4 mm.

Population dynamics in artificially infested soil. The number of propagules in artificially infested soil with or without fruit remained relatively constant during the first 6 mo of the study (September–February 1987) (Fig. 2). After that time, the number of propagules gradually declined in infested soil without fruit added. However, the number of propagules increased significantly from March to June 1988 in soil adjacent to decayed fruit. At no time, however, was *P. malorum* recovered from decaying fruit in the infested soil or from fruit decaying on the orchard floor at the soil sampling sites. Several types of unidentified fungi and bacteria as well as *Alternaria* spp., *Penicillium* spp., and *Botrytis cinerea* were isolated from the decaying fruit.

Canker development studies. Cankers consistently developed when mycelium of *P. malorum* was inserted into wounded bark. Canker development was slow, with most advancement occurring during the spring after inoculation (Table 1). Cankers did not develop when sterile agar was inserted into wounded bark, but bark adjacent to wounds frequently died back 1–4 mm. Cankers

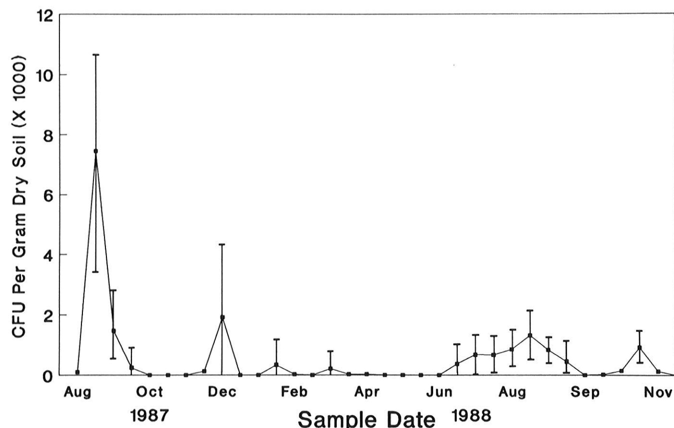

Fig. 1. Populations of *Phialophora malorum* in soil of a pear orchard near Medford, OR. Values were calculated from the number of colony-forming units (CFU) on a semiselective medium after dilution plating. Values represent means of 10 sampling sites in the drip line of randomly selected Bosc pear trees. Vertical bars indicate standard errors of means.

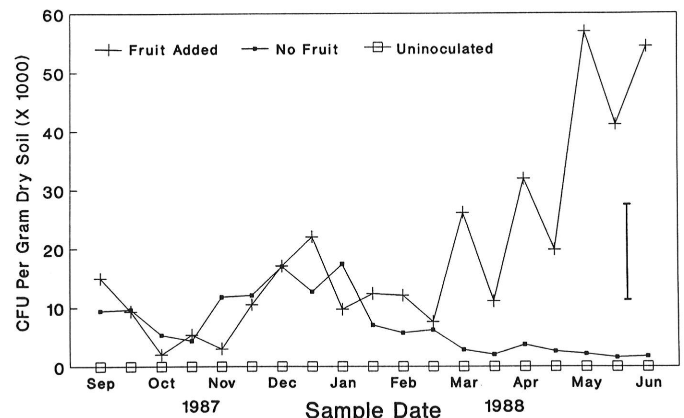

Fig. 2. Populations of *Phialophora malorum* in artificially infested soil with and without addition of pear fruit. Nonsterile potting soil in 3.79-L pots was mixed with 100 ml of a spore suspension of *P. malorum* (10^5 conidia per milliliter) and maintained outdoors during a 9-mo sampling period. Control pots were noninfested. A single mature Bosc pear was half-buried in one half of the infested pots. Values were calculated from numbers of colony-forming units (CFU) on a semiselective medium after dilution plating. Values represent the means of nine replicate pots. Vertical bar indicates least significant difference ($P = 0.05$).

TABLE 1. Growth of cankers on Bosc pear trees induced by inoculation with mycelium of *Phialophora malorum* into wounded bark

Inoculation date	Mean net canker length (mm)[a]										
	1987				1988					1989	
	Aug.	Sept.	Oct.	Nov.	Jan.	Mar.	May	July	Sept.	Jan.	July
1987											
July	4.2	5.4	5.8	9.0	8.2	8.4	10.6	8.4	10.0	9.8	9.4
Aug.	...	0.4	0.8	1.8	1.4	1.6	1.4	2.0	3.0	2.4	3.0
Sept.	...	...	0.0	0.0	0.6	1.0	1.4	1.2	1.0	1.8	1.8
Oct.	...	...	...	0.0	0.0	0.0	1.2	2.0	3.8	3.0	2.6
Nov.	...	...	...	...	0.4	2.0	11.4	11.2	13.4	13.0	13.4
Dec.	...	...	...	...	0.0	1.2	9.4	12.0	13.4	13.6	13.8
1988											
Jan.	...	...	...	...	...	0.0	6.8	7.0	7.6	6.8	7.0
Feb.	...	...	...	...	...	0.0	3.2	3.2	4.4	3.2	3.8
Mar.	...	...	...	...	...	...	6.6	6.4	7.0	6.2	6.0
Apr.	...	...	...	...	...	...	0.6	0.4	0.4	0.4	2.6
May	...	...	...	...	...	...	...	1.2	1.4	2.2	2.2
June	...	...	...	...	...	...	...	1.4	2.2	2.8	6.2
July	...	...	...	...	...	...	...	...	2.6	9.0	9.4
Aug.	...	...	...	...	...	...	...	...	0.8	0.6	7.6
Sept.	...	...	...	...	...	...	...	...	...	1.4	0.6
Oct.	...	...	...	...	...	...	...	...	...	0.2	3.8
Nov.	...	...	...	...	...	...	...	...	...	0.2	5.6
Dec.	...	...	...	...	...	...	...	...	...	0.0	2.6

[a] Disks of bark 10 mm in diameter were removed and replaced with disks of mycelium of *P. malorum* grown on potato-dextrose agar. Control treatments received disks of sterile agar. Net length was determined by subtracting mean length of wounds at control treatment sites from mean length of cankers at inoculation sites.

TABLE 2. Recovery of *Phialophora malorum* from cankers in Bosc pear trees inoculated by mycelial mats inserted into wounded bark

Inoculation date	*P. malorum* isolated from canker margin[a]				
	Apr.	June	Aug.	Oct.	Dec.
1987					
Sept.	+	+	−	−	−
Sept.	+	+	−	−	−
Nov.	+	+	+	−	−
Nov.	+	−	−	−	−
Dec.	+	+	+	−	−
Dec.					
1988	+	+	+	−	−
Feb.	+	+	+	−	−
Feb.	+	+	−	−	−
Mar.	+	+	+	−	−
Mar.	+	+	+	−	−

[a] All isolations were in 1988. + = *P. malorum* isolated from canker, − = *P. malorum* not isolated from canker. Isolations were made from one canker developing from inoculations at each of the listed dates.

were nonperennial. Canker elongation generally ceased after one season of growth, and host tissue was regenerated in cankered and wounded areas. Isolations through 1988 from the margins of cankers induced by inoculations with mycelium indicated the presence of *P. malorum* in spring and early summer, but *P. malorum* was isolated less frequently in late summer and was not recovered in October or December (Table 2). At no time was *P. malorum* isolated from suspected cankers in the orchard where soil population studies were conducted.

Cankers did not develop on 1-, 2-, or 3-yr-old wood when *P. malorum* spore suspensions were held in contact with uninjured bark. However, in inoculations made from December 1986 through June 1987 using plastic tubes, the bark was inadvertently injured by pressure placed on the tubes during inoculation, and cankers frequently developed at these wound sites. No cankers developed in the water controls. *P. malorum* was consistently recovered in washes of cankers that developed after mycelial inoculation (Table 3).

Survival on bark. *P. malorum* was recovered for more than 1 yr in washes from branch sites that were infested by spore suspension at which cankers had not developed (Fig. 3). The fungus was recovered from sites that had been infested at all

TABLE 3. Number of propagules of *Phialophora malorum* recovered in washes of cankers on Bosc pear trees

Sample date	Number of colonies per milliliter[a]	Standard deviation
1988		
28 June	86.0	63.2
26 July	38.4	16.6
18 Aug.	57.2	78.0
26 Aug.	92.8	85.6
08 Sept.	74.8	79.0
23 Sept.	110.4	71.0
07 Oct.	306.4	86.4
20 Oct.	221.6	83.6
15 Nov.	94.8	60.2
29 Nov.	431.2	216.6
15 Dec.	292.0	270.6
1989		
04 Jan.	108.8	69.0
20 Jan.	120.8	78.4
31 Jan.	182.4	37.1
16 Feb.	172.0	90.6
15 Mar.	70.4	47.4
20 Apr.	56.8	70.8
17 May	132.8	128.6
14 June	38.8	29.2
11 July	80.8	39.4

[a] Mean of five cankers. Cankers developed following artificial inoculation with mycelium of *P. malorum* in 1987.

inoculation dates except that site infested in October 1987. The number of propagules recovered in washes made in September declined sharply as time from infestation increased from 2 wk to 3 mo. *P. malorum* was recovered from bark infested at all dates in washes made in October–December 1988 (data not shown). *P. malorum* was not recovered from washes of sites of control inoculations.

DISCUSSION

The recovery of *P. malorum* from orchard soil over a 2-yr period indicates that the fungus is resident in soil and that soil may serve as a source of inoculum for postharvest fruit decay.

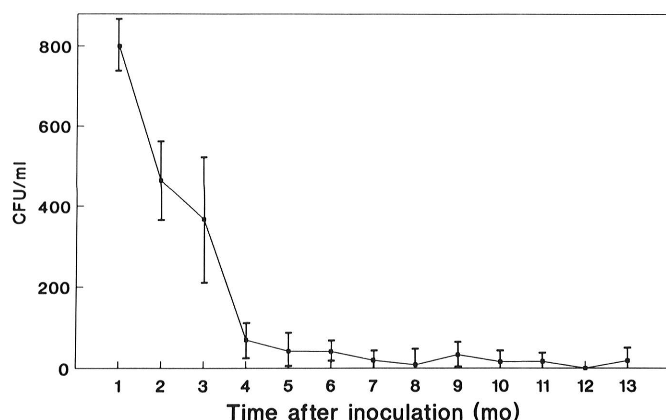

Fig. 3. Survival of *Phialophora malorum* on 3-yr-old bark of Bosc pear trees after artificial infestation with spores. Inoculation sites were washed in September 1988, 2 wk after the final infestation. Data points represent mean number of colony-forming units (CFU) recovered from one inoculation site on each of five trees. Vertical bars indicate standard errors of means.

This is further supported by the slow rate of decline of propagule numbers over a 9-mo period in artificially infested soil in pots. However, addition of fruit to infested soil did not increase propagule levels until the fruit were substantially decayed, approximately 6 mo after introduction into the soil. This population response and the lack of recovery of *P. malorum* from the decaying tissue indicate that *P. malorum* uses nutrients released into the soil by other microorganisms breaking down tissues of fallen pear fruit and, possibly, by other organic matter sources. This pattern contrasts with that of *Mucor piriformis* E. Fisch., also a postharvest pathogen of pear, which was shown to increase in soil rapidly in the presence of pear fruit on the orchard floor and to be a frequent primary colonizer of pear tissue (3). Furthermore, failure to recover *P. malorum* from fruit decaying in an environment free of imposed selective chemical agents supports the observation of Sugar and Powers (13) that *P. malorum* is a relatively weak competitor in colonization of pear tissue during cold storage.

Since the number of propagules of *P. malorum* in soil supplemented with pear fruit increased near the end of the experiment (Fig. 2), it is not clear how long relatively high populations of the fungus would be sustained by nutrients released from decayed fruit, or when a maximum population level would be reached. Under orchard conditions, use of nutrients released from decaying fallen fruit by *P. malorum* is likely to be influenced by the particular soil flora present, by environmental factors such as temperature and moisture, and by the rate of contact between released nutrients and propagules of *P. malorum*. Although the distribution of propagules of *P. malorum* was not related to a position relative to tree trunks, propagule distribution may be related to the positions of decayed fallen fruit on the orchard floor, as demonstrated for *M. piriformis* (4).

Successful induction of cankers in Bosc pear trees by inoculation with *P. malorum* was always associated with bark injury. Whereas bark injuries may occur in orchards after events such as insect damage, limb breakage, or pruning, the inability of *P. malorum* to infect through intact bark, taken together with the relatively brief persistence of the fungus in induced cankers, makes it unlikely that canker development plays a significant role in the side rot disease cycle. Furthermore, *P. malorum* was not recovered in isolations from potential cankers in the orchard where soil population studies were conducted.

The period of rapid canker development observed in the spring after inoculation during the previous summer or fall (Table 1) suggests that this is a period of heightened susceptibility in the pear tree. Alternatively, temperatures could be particularly favorable for fungus development during the spring, although Gardner (6) reported colony growth of *P. malorum* on PDA at 12–34 C.

Although *P. malorum* was recovered in washes of induced cankers, propagule recovery did not diminish after the fungus could not be detected in isolations from cankered tissue. This indicates that superficial populations may be a more critical source of propagules than sporulation from cankers per se. Recovery of *P. malorum* from noncankered bark sites up to 1 yr after inoculation by spore suspension indicates that such survival is possible, and superficial populations also may serve as sources of inoculum. However, nonrecovery at control sites on neighboring branches over the same period indicates that such superficial colonization may not be widespread. In addition, related experiments (12) showed that fruit were not infested with *P. malorum* during the growing season, indicating that spores, if present on the bark, are not readily transferred to the fruit.

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