The Importance of Wounds in Infection of Pear Fruit by *Phialophora malorum* and the Role of Hydrostatic Pressure in Spore Penetration of Wounds

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ABSTRACT

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Pear fruits (cv. Bosc) were not infected by *Phialophora malorum* via lenticels under conditions of bruising, hydrostatic pressure, or 6 mo of exposure to a range of spore concentrations without puncture wounding. Methylene blue solution penetrated approximately 2% of fruit lenticels examined. Dye penetration into fruit was facilitated by artificial bruising of lenticels. Hydrostatic pressure from immersion of fruit in aqueous suspensions of *P. malorum* spores at depths from 10 to 100 cm influenced the frequency of infection in fruit puncture wounds <2 mm in diameter. Larger wounds were infected independently of immersion depth. Germination of spores of *P. malorum* was stimulated in water in which wounded fruit or fruit with epicuticular wax removed had been soaked, as compared to water in which whole fruit had been soaked.

Additional keywords: blue mold, *Penicillium expansum*, postharvest decay, *Pyrus communis*.

Side rot, caused by *Phialophora malorum* (M.N. Kidd & A. Beaumont) McColloch is an important postharvest disease of pear in southern Oregon (6,7). It is a disease of pears in long-term storage; it rarely is observed before 3 mo at −1 C and more commonly appears 4–6 mo after fruit is placed in cold storage (31). Side rot is not controlled by any fungicide currently registered for postharvest use on pear (7,30). *P. malorum* resides in orchard soil (31) and may be transported into packinghouses on fruit surfaces or in soil adhering to harvest bins (32). In southern Oregon, most pears are harvested into wooden bins holding approximately 500 kg of fruit. At the packinghouse, fruit are floated out of bins after immersion in tanks of water containing a flotation salt and sodium-*o*-phenylphenate as a disinfestant (13,18,28). Immersion tanks often harbor spores of decay fungi (8,9,25,27) and may be important sites of redistribution of *P. malorum* inoculum in pear (32). When fruits in bins are immersed in packinghouse dump tanks, fruits at different locations in the bin are exposed to hydrostatic pressure corresponding in part to the height of solution above them, ranging from a few centimeters to approximately 1 m.

Bertrand et al (7) observed that *P. malorum* can penetrate apparently unbroken skin of pear fruit and demonstrated this at 10 and 20 C but not at colder temperatures. Pears are stored commercially at −1.1 to 0 C (21,23). Several studies of *P. malorum* as the causal agent of postharvest decay of apple have noted that lesions tend to be centered around lenticels or small wounds (14,15,17,19). However, McColloch (20) noted that it is difficult to determine whether the point of entrance is through a lenticel or through cuticular cracks. *Penicillium expansum* Link also has been reported to infect via lenticels as well as wounds (1,2,4).

The objectives of this study were to evaluate the roles of lenticels and wounds in infection of pear fruit, and the importance of hydrostatic pressure in immersion tanks on penetration of fruit by *P. malorum*.

MATERIALS AND METHODS

Lenticels as infection courts. The ability of *P. malorum* to infect pear fruit via lenticels was tested by infectivity titration. Plastic tubes approximately 6 × 4 mm (length/internal diameter) were coated on the bottom edge with petroleum jelly and centered around lenticels on the surfaces of pear fruits (cv. Bosc) stabilized on cupped fiberboard trays. A spore suspension was prepared by washing the surface of a 4-wk-old colony of *P. malorum* growing on potato-dextrose agar (PDA), and the spore concentration was adjusted with the aid of a hemacytometer. The tubes were filled with 0.1 ml of distilled water containing either 0, 10, 10^2, 10^3, 10^4, 10^5, or 10^6 conidia of *P. malorum* per tube. Tubes were placed over lenticels on 25 pears for each spore concentration. To verify the infectivity of the inoculum, five additional fruits at each inoculum concentration were punctured with a dissecting needle inserted through the spore suspension in the tubes, opening a wound approximately 0.75 mm in diameter × 2 mm deep. The trays were covered with polyethylene bags and held at 0 C. The fruits were examined periodically for development of decay over a 6-mo period. The experiment was repeated the following year.

To evaluate the permeability of Bosc pear lenticels, 25 fruits were soaked for 24 h in water containing 250 mg of methylene blue per liter (11) and then rinsed in fresh water. Thin sections were cut through lenticels into fruit cortical tissue and were examined microscopically to determine the extent of dye penetration via the lenticel. A total of 185 lenticels were examined.

The effect of bruising on permeability of lenticels and interlenticellular epidermal areas was examined by dropping a 142-g round-head steel bolt through 1.9-cm-diameter plastic tubes 5, 10, 15, or 20 cm long centered over lenticels or interlenticellular epidermal areas on each of 10 Bosc pears per treatment. Fruits were then soaked for 24 h in a methylene blue solution, as above, and examined microscopically for dye penetration. The experiment was performed twice.

Effect of hydrostatic pressure on lenticel or wound penetration. Experiments were designed to investigate the role of hydrostatic pressure in the immersion tank on penetration of fruit by spores of decay fungi. A vertical plastic tube 110 cm high × 10 cm in diameter was filled with water to 100 cm. A device to lower fruit into the tube was fashioned by attaching a perforated 8-cm-diameter plastic disk to one end of a 125-cm wooden dowel, with a wire-mesh basket large enough to carry one Bosc pear on the underside of the disk. The dowel was marked in 10-cm increments from the plastic disk to 100 cm. The perforated plastic disk, in addition to providing an attachment point for the mesh

basket, ensured thorough mixing of the spore suspension in the tube as the dowel was raised or lowered.

In one set of experiments, spores were washed from a 4-wk-old colony of *P. malorum* growing on PDA and added to water in the tube to a final concentration of 10^5 spores per milliliter, determined with a hemacytometer. Bosc pears were surface-disinfested for 5 min in 0.5% sodium hypochlorite (NaOCl) and puncture-wounded to a depth of 2 mm with steel needles or steel finishing nails to give wound diameters of 0.4, 0.5, 1, 2, or 6 mm. Fifteen unwounded fruits and fruits with each size wound were individually immersed for 15 s, 2 min, or 5 min in the spore suspension at depths from 0 to 100 cm in 10-cm increments. Each fruit was rinsed for 15 s in tap water and stored in polyethylene-lined boxes at 0 C. After 4 mo, each fruit was examined for decay at wound sites, and isolations from lesion tissue on PDA were made to confirm *P. malorum* as the causal agent. *P. malorum* was identified by colony morphology and color and by microscopic examination of the shape and size of conidiogenous cells and conidia (20). For comparison, the experiment was repeated with the tube water containing conidia of *Penicillium expansum*, causal agent of blue mold decay of apple and pear (1,3,12,22), immersed to depths of 0, 20, 40, 60, 80, and 100 cm.

Lenticels of Bosc pears were bruised by dropping a weight from 0, 5, 10, 15, or 20 cm as described above and were immersed in the *P. malorum* spore suspension to depths of 0, 10, 20, 40, 60, 80, or 100 cm. All fruits were stored for 4 mo at O C and evaluated as above.

Effect of wound exudates on spore germination. An experiment was conducted to evaluate whether wounds in pear fruit, in addition to being an infection court for *P. malorum*, provide stimulus for spore germination. Five Bosc pears, surface-disinfested in NaOCl, were wounded with a sterile 6-mm-diameter nail to a depth of 3 mm. The fruits then were individually soaked for 18 h in 250 ml of distilled water. After removing the fruit from the water, 1 ml of a spore suspension of *P. malorum* (10^4 per milliliter) was added to the water, and the solution was incubated at 15 C. After 2, 4, 6, and 8 days of incubation, five 0.1-ml aliquots were removed from the solutions and examined microscopically to determine the percentage of spores that had germinated. Spores were considered germinated if germ tubes were greater than one-half the length of the spore (30). The same procedure also was carried out with unwounded fruit and fruit from which extracuticular wax had been removed by a 20 s dip in chloroform (24), followed by a fresh-water rinse. Spores of *P. malorum* were added to distilled water in which fruit had not been soaked. The total soluble solids (degrees Brix) of each soaking solution was determined with a hand-held refractometer (Model 10421, American Optical Co., Keene, NH). The experiment was performed twice.

TABLE 1. Infection of Bosc pears at lenticels by spores of *Phialophora malorum* at various inoculum concentrations

Inoculum concentration (spores/0.1 ml)	Number of lenticels infected[a]	
	Unwounded (25 fruits)	Punctured (5 fruits)
0	0	0
10	0	2
10^2	0	5
10^3	0	5
10^4	0	5
10^5	0	5
10^6	0	5

[a] Plastic cylinders were centered over lenticels, sealed to fruit surfaces with petroleum jelly, and filled with 0.1 ml of *P. malorum* spore suspension. Five lenticels were punctured through the lenticel with a dissecting needle to a depth of 2 mm. Infection was evaluated monthly during 6 mo of incubation at 0 C. Results were identical when the experiment was repeated.

TABLE 2. Penetration of lenticels or interlenticellular (IL) areas in Bosc pears by a methylene blue solution after bruising

Drop height[a] (cm)	Penetration	
	Lenticels (%)	IL areas (%)
0	0.0	0.0
5	0.0	0.0
10	0.0	0.0
15	20.0	0.0
20	65.0	15.0

[a] A 142-g round-head steel bolt was dropped through tubes of various lengths onto marked fruit lenticels or IL areas prior to soaking fruit 24 h in methylene blue solution at 250 mg/L. Penetration was determined by the observation of blue color in parenchymatous tissue in thin section. Data from two repetitions of the experiment were combined.

RESULTS

Lenticels as infection courts. Infection of Bosc pears via lenticels was not observed in any of the experiments in which intact lenticels were exposed to inoculum of *P. malorum*. Inoculum held in contact with lenticels for 6 mo did not result in lesion development, but lesions developed at wounds in fruit receiving the same inoculum treatments (Table 1). Of the 185 lenticels dissected after dye treatment, only four were "open," as indicated by dye penetration of parenchymatous tissue beneath the lenticel. Bruising of lenticels increased penetration by dye solution, and dye penetrated lenticels at a lower impact than in interlenticellular epidermal areas (Table 2). *P. malorum* did not penetrate fruit lenticels at any level of hydrostatic pressure tested, regardless of bruising.

Effect of hydrostatic pressure on wound penetration. Infection of Bosc pears by *P. malorum* was affected by an immersion depth–wound size interaction (Fig. 1). Exposure time (15 s, 2 min, or 5 min) did not affect wound infection significantly ($P > 0.05$). Infection did not take place at any depth in unwounded fruit. At wound diameters of 0.4 and 0.5 mm, infection occurred in 0–20% of the wounds, and infection increased with greater

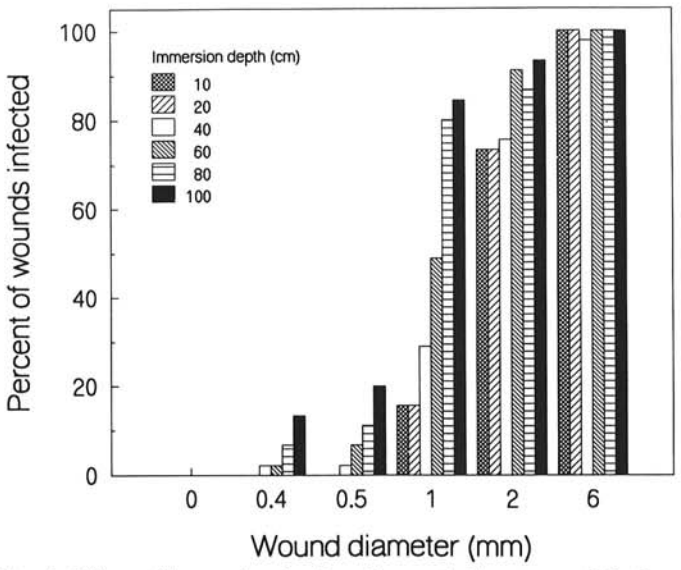

Fig. 1. Effects of immersion depth and wound diameter on infection of pear fruit by *Phialophora malorum* after immersion in water containing 10^4 conidia per milliliter. Fruits were held at indicated depths for 15 s, 2 min, or 5 min (data combined), rinsed in fresh water, and stored for 3 mo at 0 C prior to evaluation of lesion development. Regression analysis was performed separately within each wound-diameter category. No significant relationship existed between immersion depth and infection at 6-mm wound diameter ($P > 0.05$). Regression of percent wound infection on immersion depth with wound diameters of 0.4, 0.5, 1, and 2 mm were described by the following equations: $Y_{0.4} = -3.10 + 0.137\ X$, $R^2 = 0.56$, $P < 0.001$; $Y_{0.5} = -3.84 + 0.199\ X$, $R^2 = .69$, $P < 0.001$; $Y_1 = -3.24 + 0.924\ X$, $R^2 = .83$, $P < 0.001$; and $Y_2 = 57.63 + 0.403\ X$, $R^2 = 0.49$, $P < 0.001$.

immersion depth. At wound diameters >1 mm, infection took place at all immersion depths, and infection occurred in 68–100% of the wounds. Infection of wounds 1 mm in diameter was influenced strongly by immersion depth, whereas the effect of immersion depth was not significant in wounds 2 or 6 mm in diameter according to linear regression analysis (Fig. 1). A similar relationship between wound size and immersion depth was observed using spores of *Penicillium expansum* as inoculum (Fig. 2).

Effect of wound exudates on spore germination. Percent spore germination and germination speed were significantly greater in water in which wounded fruit had been soaked than in water alone or water in which unwounded pears had been soaked (Fig. 3). After 2 days, 32.0% of the spores had germinated in water in which wounded fruit had been soaked, whereas less than 5% of the spores in all other treatments had germinated at this time. Soaking of fruit with wax removed stimulated spore germination to a greater extent than water alone or water in which whole fruit had been soaked, but to a lesser extent than water in which wounded fruit had been soaked. All treatment solutions had Brix values of 0°, indicating that the concentration of dissolved sugars was not detectably increased by the treatments.

DISCUSSION

It is difficult to say unequivocally whether *P. malorum* is capable of entering fruit via lenticels. Studies reporting infection of apples by *P. malorum* via lenticels have considered absence of macroscopically visible skin breaks and apparent centering of lesions about lenticels as evidence that lenticels function as infection courts. In the experiments reported here, lenticel infection was not induced in Bosc pears, despite conditions of high inoculum concentration, prolonged contact between lenticels and inoculum, bruising, and hydrostatic pressure during immersion infestation. Because most lenticels sampled in this study were not open to dye penetration, it is possible that lenticel condition plays a determining role in frequency of lenticel infection. Lenticel condition may be a function of species, cultivar, and climatic or cultural conditions (11). Because bruising more effectively induced dye penetration at lenticels than at interlenticellular areas, lenticels could be more vulnerable to infection than other epidermal areas under certain conditions. Clements (11) examined methylene blue penetration of lenticels in 13 apple cultivars and found that the proportion of open lenticels varied with cultivar from 4.8 to 29.8%. English et al (14) dissected blue mold (*P. expansum*) lesions in Delicious apples apparently initiated via lenticels and concluded that 63% of the lesions examined were true lenticel infections. However, although also noting lenticel infection of Delicious apples by *P. malorum*, they did not further examine side rot lesions. They also observed that a large majority of blue mold infections in commercial apples were found at wounds of various sizes. Although Bertrand et al (7) reported successful induction of infection of Bosc pears by *P. malorum* via lenticels after 3 wk at 20 and 10 C at an inoculum dose of 10^6/ml, the percentage of lenticels infected was 33 and 40%, respectively, further suggesting a differential condition of lenticels. A factor that may have influenced their results, however, is the fact that Bosc pears ripen and cell senescence may be advanced within 3 wk at incubation temperatures of 20 or 10 C (16,21,23); as a result, lenticel susceptibility after 3 wk at these temperatures may not be indicative of lenticel susceptibility under commercial storage conditions at −1 to 0 C, as simulated in this study.

Bosc pears are prone to mechanical injury and have sharp-pointed vegetative and flower buds that may increase incidence of fine punctures in the fruit during harvest or in wind-driven movement of fruits on the tree (29). The fruits do not roll well on packinglines due to their elongated shape, and frequent impact with stems of adjacent fruit may result in small punctures. Although experiments in this study showed that infection of smaller wounds is less likely than infection of larger wounds, 13.3 and 20%, respectively, of wounds 0.4 and 0.5 mm in diameter were infected after immersion to 100 cm. The percentage of infection may be increased with greater hydrostatic pressure due to depth in the immersion tank. Results of a packinghouse survey conducted during 1988 demonstrated that wounds of various sizes occur during commercial handling of pears, and that puncture wounds are the most important cause of fruit cullage (D. Sugar, *unpublished data*). Small wounds are particularly difficult to detect in Bosc pears during packinghouse sorting and cullage due to the dark background skin color and irregular russet patterns on the fruit surface (29).

Exudation via wounds stimulates spore germination in *P. malorum* and may affect the occurrence of infection at wound

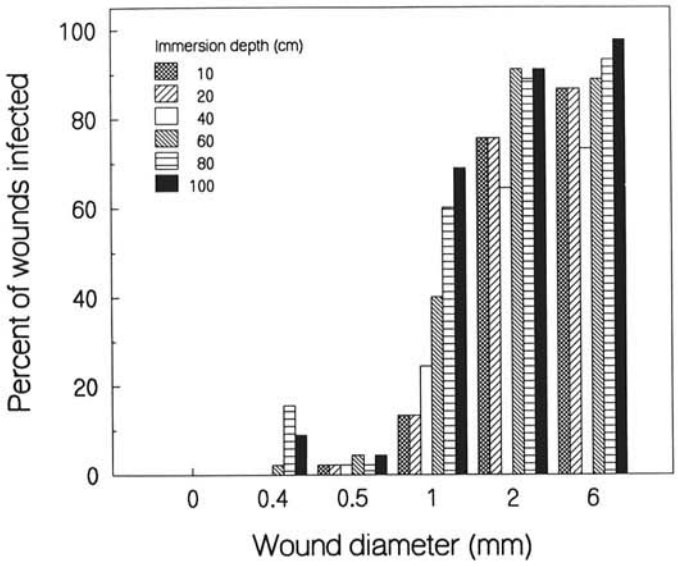

Fig. 2. Effects of immersion depth and wound diameter on infection of pear fruit by *Penicillium expansum* after immersion in water containing 10^4 conidia per milliliter. Fruits were held at indicated depths for 15 s, 2 min, or 5 min (data combined), rinsed in fresh water, and stored for 3 mo at 0 C prior to evaluation of lesion development. Regression analysis was performed separately within each wound-diameter category. No significant linear relationships existed between immersion depth and infection at 0.5- or 6-mm wound diameters ($P > 0.05$). Regression of percent wound infection on immersion depth with wound diameters of 0.4, 1, and 2 mm were described by the following equations: $Y_{0.4} = -3.107 + 0.146\ X$, $R^2 = 0.31$, $P = 0.016$; $Y_1 = 1.851 + 0.675\ X$, $R^2 = 0.61$, $P < 0.001$; and $Y_2 = 60.06 + 0.35\ X$, $R^2 = 0.44$, $P = 0.003$.

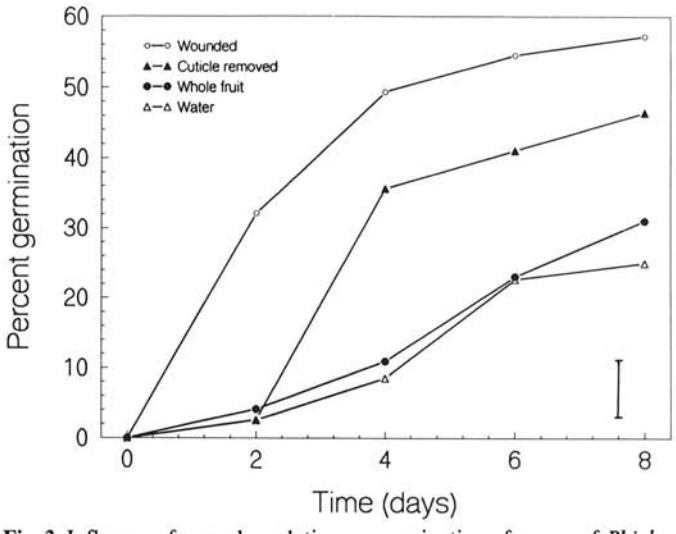

Fig. 3. Influence of wound exudation on germination of spores of *Phialophora malorum*. Bosc pears were either unwounded, wounded with a finishing nail 3 mm deep × 6 mm in diameter or had epicuticular wax removed by a 20 s dip in chloroform. Fruits then were soaked 18 h in 250 ml of distilled water. After fruits were removed, 1 ml of a spore suspension of *P. malorum* was added to the water and to a distilled water control, and spore germination was evaluated over 8 days at 15 C. Bar indicates LSD ($P = 0.05$).

sites. Although the nature of the stimulus was not determined, leakage of nutrients at levels below detection by hand-held refractometer may be sufficient to influence spore germination. Stimulation of spore germination also may be due to leakage of substances that require methods other than refractometry to detect.

Based on the relationship between wound size and immersion depth (Figs. 1 and 2), we suggest that their interaction is based on the amount of force required for the spore suspension to penetrate the wound cavity. Infection of wounds 6 mm in diameter by *P. malorum* was independent of immersion depth, whereas infection of wounds $\leq$1 mm in diameter generally depended on immersion depth. Increased penetration of apple cores by dip solutions, and a consequently greater risk of core rot, is associated with increasing immersion depths (26). Hydrostatic pressure in immersion tanks also has been shown to increase infiltration of *Erwinia carotovora* into lenticels of potato tubers (5).

A static load of 100 cm of water is equivalent to a pressure of 0.09869 atm or 9.806 kPa (10). The precise relationship between wound size, immersion depth, and fruit penetration in a fruit immersion tank may be influenced by the presence of dissolved and suspended materials that affect the specific gravity and surface tension of the solution (8). However, based on the results of this study, depth of immersion may be a significant factor in the incidence of fruit infection by postharvest decay fungi.

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