AN ABSTRACT OF THE THESIS OF

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Title: The Disease Cycle of Side Rot of Pear, Caused by Phialophora malorum.

Abstract approved: Redacted for privacy

Robert A. Spotts

Potential sources of inoculum of Phialophora malorum were investigated. P. malorum was found to be a soil-borne fungus whose role as a postharvest pathogen of pear is dependent upon passive dispersal with infested soil. P. malorum survived in soil under a wide range of conditions, with greatest survival in cool, moderately dry soil of near-neutral pH. P. malorum was not a primary colonizer of fallen fruit on orchard soil, but propagule numbers increased subsequent to fruit decay by other microorganisms.

Cankers were induced when P. malorum was inoculated into injured bark of pear trees. However, cankers were non-perrenating, and P. malorum was not recovered from cankered tissue for more than one season. The fungus also survived saprophytically on the bark of pear trees, but dispersal of inoculum from infested bark to fruit or other areas of bark on the same trees was not observed.

Inoculation of fruit and redistribution of inoculum from infested to previously uninfested fruit in packinghouse immersion dump tank solutions was demonstrated. Infection took place at fruit wounds. Spore penetration into wounds was influenced by wound diameter and hydrostatic pressure during immersion dumping. Infection via intact lenticels could not be induced.

Results suggest that sanitation to avoid contamination of packinghouse immersion tanks with orchard soil may reduce disease incidence. As a weak competitor in colonizing pear tissue, P. malorum appears vulnerable to control through enhancement of fruit resistance or biological control.

# The Disease Cycle of Side Rot of Pear, Caused by *Phialophora malorum*

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Redacted for privacy

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TABLE OF CONTENTS


| | | |
|---|---|---|
| Introduction | | 1 |
| Chapter I. | Sources of Inoculum of *Phialophora malorum*, Causal Agent of Side Rot of Pear. | 3 |
| | Introduction | 3 |
| | Materials and Methods | 4 |
| | Results | 9 |
| | Discussion | 24 |
| Chapter II. | Effects of soil Temperature, pH, and Matric Potential on Population Dynamics of *Phialophora malorum*. | 30 |
| | Introduction | 30 |
| | Materials and Methods | 31 |
| | Results | 39 |
| | Discussion | 46 |
| Chapter III. | Dispersal of Inoculum of *Phialophora malorum* from Orchard Soil and Inoculum Redistribution in Pear Immersion Tanks. | 51 |
| | Introduction | 51 |
| | Materials and Methods | 52 |
| | Results | 56 |
| | Discussion | 63 |
| Chapter IV. | The Importance of Wounds as Infection Courts for Postharvest Decay of Pear by *Phialophora malorum* and the Role of Hydrostatic Pressure in Spore Penetration of Wounds | 67 |
| | Introduction | 67 |
| | Materials and Methods | 68 |
| | Results | 74 |
| | Discussion | 89 |
| Conclusions | | 95 |
| Bibliography | | 100 |

LIST OF FIGURES


| FIGURE | | PAGE |
|---|---|---|
| I-1. | Populations of Phialophora malorum in soil of a pear orchard near Medford, Oregon. | 10 |
| I-2. | Relative virulence of 45 soil isolates of Phialophora malorum. | 13 |
| I-3. | Populations of Phialophora malorum in artificially infested soil with and without addition of pear fruit. | 15 |
| I-4. | Growth of cankers on Bosc pear trees induced by inoculation of mycelium of Phialophora malorum into wounded bark. | 18 |
| II-1. | Water release curve relating soil matric potential to volumetric water content of an orchard soil mixed 2:1 with coarse sand. | 33 |
| II-2. | Water release curve relating soil matric potential to volumetric water content of an orchard soil. | 36 |

List of Figures, Continued.

II-3. Effect of temperature on survival of Phialophora malorum in a non-sterile orchard soil. 40

II-4. Effect of soil matric potential on survival of Phialophora malorum in a non-sterile orchard soil. 42

II-5. Effect of soil pH on survival of Phialophora malorum in a non-sterile orchard soil. 44

II-6. Soil temperature at Medford, Oregon at 5 cm depth from January 1987 to August 1989. 48

IV-1. Effects of immersion depth and wound diameter on infection of pear fruit by Phialophora malorum following immersion in water containing $10^4$ conidia/ml. 79

IV-2. Effects of immersion depth and wound diameter on infection of pear fruit by Penicillium expansum following immersion in water containing $10^4$ conidia/ml. 81

List of Figures, Continued.

IV-3. Influence of wound exudation on germination of spores of Phialophora malorum. 85

IV-4. Relationships between inoculum dose and disease severity in fruit of major pear cultivars wound-inoculated with spores of Phialophora malorum. 87

V-1. Diagramatic representation of the disease cycle of side rot of pear. 96

LIST OF TABLES


| TABLE | | PAGE |
|---|---|---|
| I-1. | Populations of Phialophora malorum recovered from orchard soil sampled at different locations relative to tree positions. | 12 |
| I-2. | Recovery of Phialophora malorum from cankers in Bosc pear trees inoculated by mycelial mats inserted into wounded bark. | 22 |
| I-3. | Canker development on Bosc pear trees inoculated with spore suspensions of Phialophora malorum. | 23 |
| I-4. | Number of propagules of Phialophora malorum recovered in washes of cankers on Bosc pear trees. | 25 |
| I-5. | Number of propagules of Phialophora malorum recovered from 3-yr old bark of Bosc pear trees following artificial infestation with spores of P. malorum. | 26 |
| III-1. | Recovery of Phialophora malorum from air and immersion dump tank solutions in a commercial pear packinghouse in Medford, Oregon. | 58 |

List of Tables, Continued.

III-2. Recovery of Phialophora malorum from air in a commercial orchard. 59

III-3. Recovery of Phialophora malorum from fruit surfaces in a commercial orchard. 60

III-4. Incidence of side rot in Bosc pears covered with paper bags at various times during the growing season. 61

III-5. Evaluation of latent infection in Bosc pears from an orchard with Phialophora malorum in the soil. 62

III-6. Dynamics of transfer of spores of Phialophora malorum between surfaces of Bosc pears and a circulating water bath as a simulated packinghouse immersion tank. 64

IV-1. Infection of Bosc pears at lenticels by spores of Phialophora malorum at various inoculum concentrations. 75

IV-2. Penetration of lenticels or interlenticellular areas in Bosc pears by a methylene blue solution after bruising. 76

List of Tables, continued.

IV-3. Interaction of degree of bruising and depth of immersion in a spore suspension on lenticel infection of Bosc pears by Phialophora malorum. 78

IV-4. Survey of cullage in Bosc pears from three packinghouses in Medford, Oregon. 83

IV-5. Effect of growth medium on virulence of Phialophora malorum. 90

# The Disease Cycle of Side Rot of Pear, Caused by *Phialophora malorum*

## INTRODUCTION

Side rot of pear is caused by the fungus *Phialophora malorum* (Kidd & Beaum.) McColloch. Although rare or unknown in other pear-growing regions of the world, this disease causes significant losses in stored pears in the Rogue River Valley of Oregon. In a survey of fruit and vegetable diseases on the Chicago market in 1936, Ramsey (36) observed a significant amount of decay (up to 40% of the fruit in some boxes) in Anjou pears shipped from Medford, Oregon in February. This decay was attributed to *Sporotrichum* (= *Phialophora malorum*). In a 1940 doctoral dissertation at Washington State University, English (17) noted that *S. malorum* was an infrequent pathogen of Washington pears, but mentioned anecdotally having received decayed pear specimens with a letter from F.C. Reimer of Medford in 1937, stating "[*S. malorum*] is causing considerable loss in the fruit from one orchard in this district." Forty years later (1977), Bertrand et al (7) described an epidemic of side rot in Bosc pears in the Medford district, and noted the insensitivity of the causal fungus to the fungicide benomyl, commonly applied to pears for control of postharvest decay (16).

In apple, a fungus isolated from decayed fruit in England was named Sporotrichum malorum, species novum, by Kidd and Beaumont in 1924 (25). The several prior surveys of apple rot fungi from Europe and America reviewed by Kidd and Beaumont do not include a fungus fitting the description of S. malorum. Gardner (20) described the disease and causal fungus from apples in Indiana in 1929. In 1931, Ruehle (37) identified S. malorum from apples in Washington state, and named an additional species S. carpogenum. McColloch (29) in 1942 concluded that the two species were one, and further that the species belonged in the genus Phialophora.

The above-mentioned literature noted disease occurrences, characterized lesion appearances, and described fungal features in lesions and in culture, but did not explore ecological or epidemiological aspects of the disease. The principal objective of this thesis is to elucidate aspects of the disease cycle of side rot of pear, which will then serve as a basis for the design of strategies for disease prevention or control.

# Chapter I.

## Sources of inoculum of Phialophora malorum, causal agent of side rot of pear.

### INTRODUCTION

Side rot of pear, caused by Phialophora malorum (Kidd & Beaum.) McColloch, is an important disease of stored pears in the Rogue River Valley of southern Oregon (6,7,43). Side rot is a disease of long-term stored fruit, rarely observed before 3 mo storage, and appearing more frequently after pears have been stored 4-5 mo at -1 C (43). Although characteristics of P. malorum have been described in culture and from examinations of decay lesions in apples and pears (7,17,20,25,30,37), little is known about sources of inoculum for fruit decay. McColloch (30), in a study of P. malorum as a cause of apple rot in the eastern United States, stated that the fungus lives saprophytically in surface soil, upon bark, and in cankerous woody tissue of apple trees, and that apples become infected while on the tree. He reported one isolate recovered 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 investigate sources of P. malorum providing inoculum 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 (Chapter 3). Therefore, investigations herein focused on soil, tree surfaces, and tree cankers as potential orchard sources of inoculum.

## MATERIALS AND METHODS

**Semi-selective medium.** Since P. malorum colonies develop slowly even at optimum temperatures (20,30), a semi-selective medium (SSM) was developed for enumeration of P. malorum populations in soil and on plant surfaces. The following medium proved successful in excluding most competetive organisms: 39 g potato dextrose agar (PDA)(Difco, Detroit MI); 200 mg benomyl (Benlate 50W, DuPont, Wilmington DE); 99 mg 2,6-dichloro-4-nitroaniline (Botran 75W, Upjohn, Kalamazoo, MI); 300 mg streptomycin sulfate (Agri-strep, Merck, Rahway NJ); and 41.5 mg rose bengal (J.T. Baker, Phillipsburg NJ) (28) per L distilled water. All anti-microbial 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.

**Populations of P. malorum in orchard soil.** A pear orchard which 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, Oregon. Ten mature trees were randomly chosen within the block. At 2-4 wk intervals over a 16 mo period, approximately 50 g of soil in the top 4 cm below the duff were collected from four sites around the drip line and pooled for each tree. After thorough mixing in the laboratory, 10 g of soil from each tree site were added to 90 ml distilled water in an Ehrlenmeyer flask and agitated for 20 min on a wrist-action shaker. One ml soil solution was withdrawn from each flask and diluted in 99 ml distilled water. After stirring, ten 0.5 ml aliquots of the dilution from each flask were plated on SSM. Spore washes from stock cultures of P. malorum were also spread on two SSM plates at each sampling date as a reference for colony development. After 6-8 wk incubation at 20 C, colonies of P. malorum were identified and counted on each plate. Identification was based on colony morphology and color and the shape and size of conidiogenous cells and conidia (30). Five g soil from each tree site were also dried to constant weight in an oven at 100 C and moisture content was determined. Results were expressed as the number of propagules per gram dry soil.

At three of the above tree sites, soil was sampled using the above procedure during January-February 1989 from

four locations relative to the tree: adjacent to the trunk, mid-canopy (halfway from trunk to dripline), at the dripline, and in open areas halfway between tree rows.

The relative virulence of 45 isolates of P. malorum from soil was tested by inoculating wounds in Bosc pears with 0.05 ml of a spore suspension containing $10^5$ spores/ml, and comparing lesion diameters after 3 mo incubation at 0 C.

**Population dynamics in artificially infested soil.** Twenty-seven 1-gallon plastic nursery pots were filled with non-sterile potting mix and placed on wooden benches outdoors. 100 ml of distilled water containing conidia of P. malorum ($10^5$/ml) were added to 18 of the pots and mixed thoroughly. The remaining nine pots served as uninoculated controls. In one-half of the inoculated pots, freshly-harvested mature Bosc pears 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 when necessary. At 2-4 wk intervals over a 9 mo period, approximately 5 g of soil from the surface 2.5 cm of soil in each pot was collected and populations of P. malorum 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 if 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 5 min in 0.5 % NaOCl and decayed tissue plated on PDA. Treatment data were compared by Fisher's protected least significant difference (LSD) test, using Number Cruncher Statistical System software (J.L. Hintze, Kaysville, UT).

**Canker development studies.** At monthly intervals over a 2-yr period beginning in December 1986, Bosc pear trees at the Southern Oregon Experiment Station, Medford were inoculated with P. malorum. Side rot had not previously been observed in fruit from the Experiment Station orchard. On five replicate trees, 10-mm disks of bark were removed from 3 yr-old wood and replaced with either disks of mycelium from 2-4 wk-old cultures of P. malorum growing on PDA or with sterile disks of PDA as controls. Inoculation sites were covered with parafilm (American Can Co., Greenwich CT), which was removed after 1 mo. At different locations in the same trees, 1-cm sections of plastic tubing were secured onto the bark surface of 1, 2, and 3 yr-old wood with modeling clay and filled with 0.25 ml of a spore suspension of P. malorum ($10^5$/ml) prepared by flooding 2-4 wk old colonies growing on PDA. In control treatments, tubes were filled with distilled water. All tubes were covered with parafilm for 1 mo, after which film, tubes, and clay were removed and discarded.

Canker development was measured periodically beginning in July 1987 until July 1989. Canker length was measured between the furthest points of visibly cankered tissue along the axis of the tree branch. Where cankers developed at inoculation sites, isolations were made from canker margins to determine the presence of P. malorum. Isolations were also made from 10 cankers at regular intervals during the spring and summer of 1988 to determine longevity of P. malorum in cankers. In addition, in the orchard where soil population studies were conducted, isolations were made from suspected cankers in trees at each soil sampling date.

Five cankers which developed from mycelial inoculation were washed at 2-4 wk intervals over 14 mo to enumerate spore populations. Cankers were sprayed with 0.5 ml distilled water from a hand-held atomizer, and run-off was collected and spread on SSM plates, which were incubated and evaluated as described above.

**Epiphytic survival of P. malorum.** Where cankers did not develop from spore suspensions held on the bark surface, infestation sites on 3-yr-old wood were sprayed with 0.5 ml distilled water which was collected on SSM as described above. Washes were made at monthly intervals from September-December 1988. Control sites were washed similarly. Data trends were analyzed by linear regression analysis using Statgraphics software (Statistical Graphics Corp., Rockville, MD).

# RESULTS

**Populations of *P. malorum* in orchard soil.** *P. malorum* was recovered from orchard soil by dilution plating on SSM (Fig. I-1). Although propagule levels were highly variable, the fungus was detected in the soil at most times of the year. 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. *P. malorum* was recovered from all four locations relative to the trees, although soil populations were not high during the sampling period (Table I-1). All isolates evaluated for virulence caused lesion development on Bosc pears. Mean lesion diameters after 3 mo incubation ranged from 11.6-14.4 mm (Fig. I-2).

**Population dynamics in artificially infested soil.** The number of propagules in artificially infested soil remained relatively constant during the first 6 mo of the study (Fig. I-3). After that time, the number of propagules gradually declined in infested soil without fruit added. However, the number of propagules increased significantly in soil adjacent to decayed fruit. At no time, however, was *P. malorum* recovered from decaying fruit in the infested soil, nor from fruit decaying on the orchard floor at the soil sampling sites. Several types of unidentified fungi and

Fig. I-1. Populations of Phialophora malorum in soil of a pear orchard near Medford, Oregon. Values were calculated from the number of colonies forming on a semi-selective medium following 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.

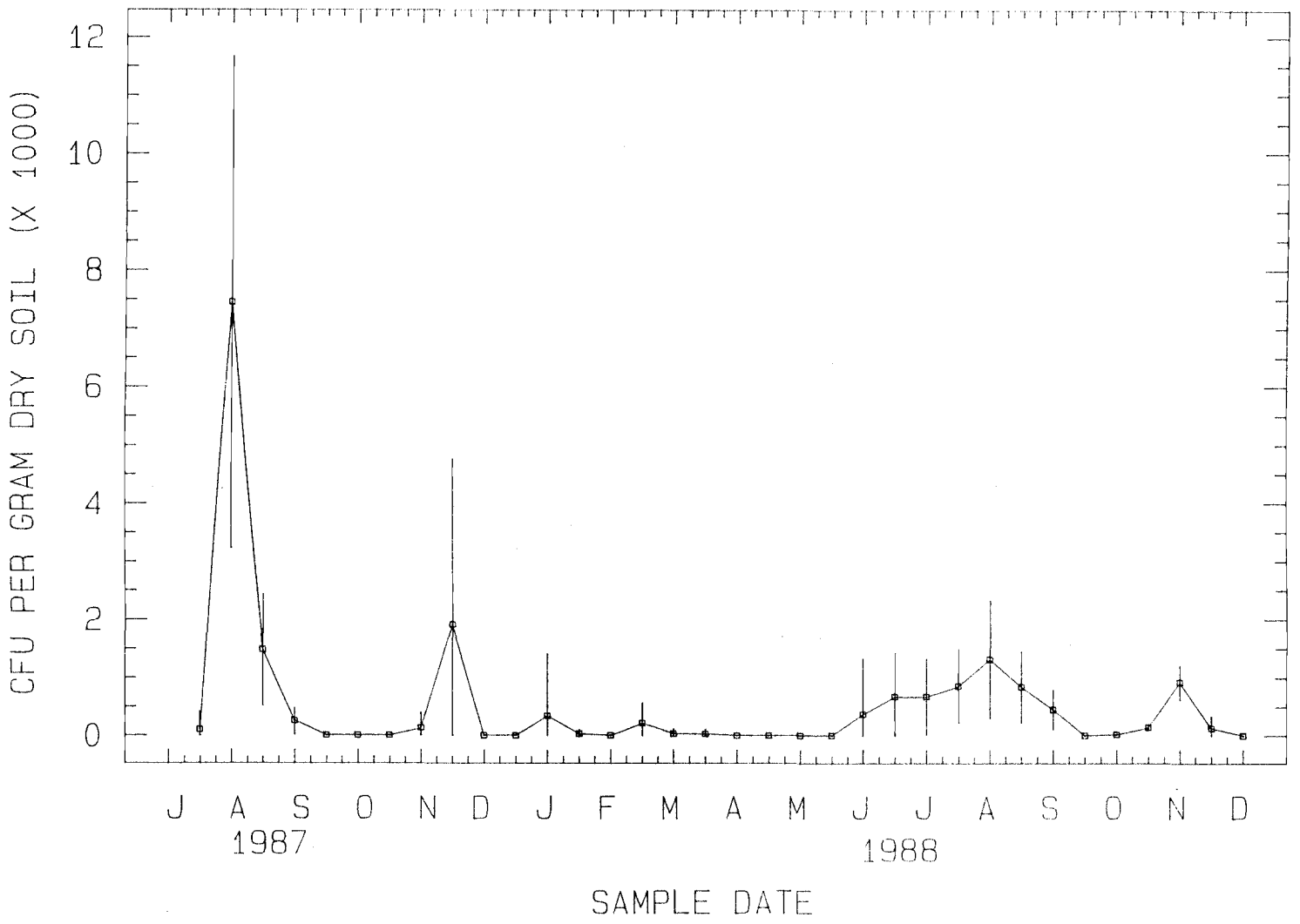
CFU PER GRAM DRY SOIL (X 1000)
12
10
8
6
4
2
0
J A S O N D J F M A M J J A S O N D
1987
1988
SAMPLE DATE

Fig. I-1.

Table I-1. Populations of Phialophora malorum recovered from orchard soil sampled at different locations relative to tree positions.

| | CFU per gram dry soil[a] | | | |
|---|---|---|---|---|
| Sample date | Adjacent to trunk | Mid-canopy | Dripline | Between trees |
| 01/17/89 | 156.9 | 78.4 | 0.0 | 74.9 |
| 01/28/89 | 78.4 | 0.0 | 76.6 | 306.5 |
| 02/10/89 | 0.0 | 0.0 | 153.2 | 0.0 |
| 02/24/89 | 0.0 | 0.0 | 0.0 | 0.0 |

[a] Each value was calculated from the mean number of colonies per plate growing on ten plates from each of three replicates.

Fig. I-2. Relative virulence of 45 isolates of Phialophora malorum from soil. Wounds in Bosc pears were inoculated with 0.05 ml of a suspension containing $10^4$ conidia of P. malorum per ml. Lesion diameters were measured after 3 mo storage at 0 C. Values represent means of 25 lesions of each isolate.

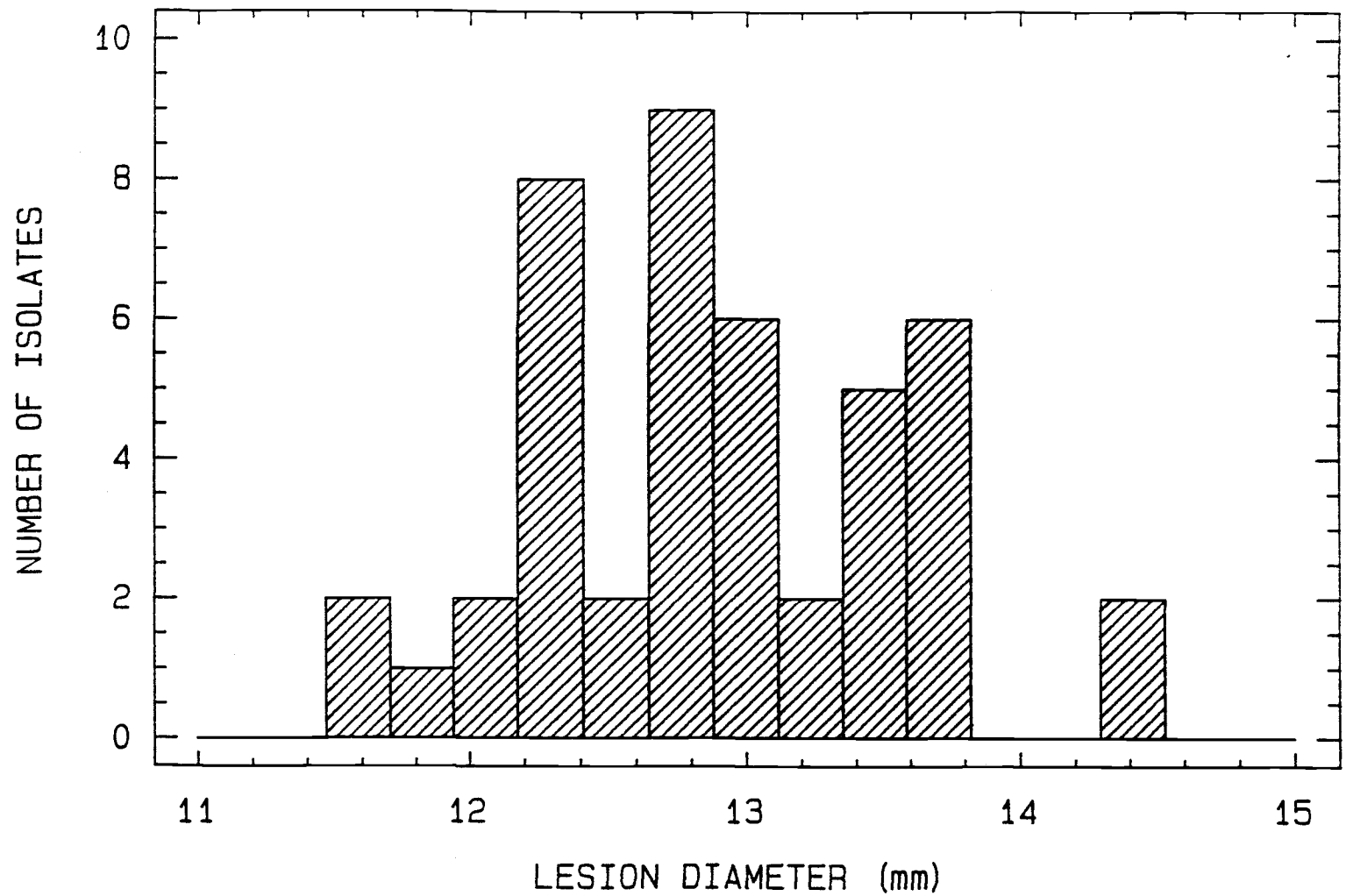


Fig. I-2.

Fig. I-3. Populations of Phialophora malorum in artificially infested soil with and without addition of pear fruit. Non-sterile potting soil in 1 gallon pots was mixed with $10^6$ conidia of P. malorum and maintained outdoors during a 9 mo sampling period. Control pots were uninfested. A single mature Bosc pear was half-buried in one-half of the infested pots at the beginning of the experiment. Values were calculated from numbers of colonies forming on a semi-selective medium following dilution plating. Values represent the means of 9 replicate pots. Vertical bar indicates LSD ($P$=0.05).

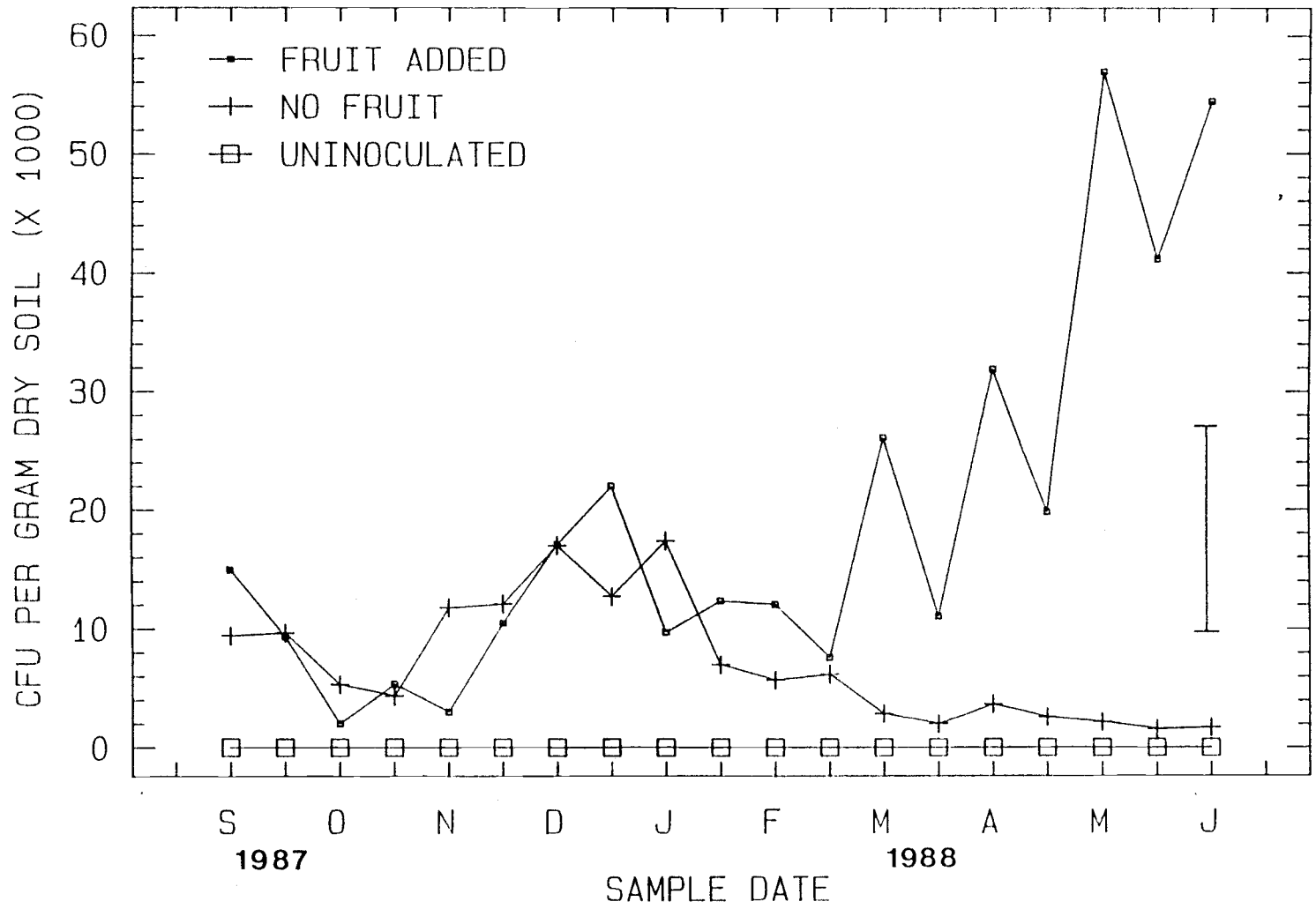
FRUIT ADDED
NO FRUIT
UNINOCULATED
CFU PER GRAM DRY SOIL (X 1000)
60
50
40
30
20
10
0
S
O
N
D
J
F
M
A
M
J
1987
1988
SAMPLE DATE

Fig. I-3.

bacteria as well as Alternaria spp., Penicillium spp., and Botrytis cinerea Pers.: Fr. 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 advancement occuring primarily during the spring following inoculation (Fig. I-4). Cankers did not develop when sterile agar was inserted into wounded bark, but bark adjacent to wounds frequently died-back 1-4 mm. Perrenation of cankers did not occur, as 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 in the fall and early winter of 1987 indicated the presence of P. malorum in spring and early summer, but P. malorum was isolated less frequently in late summer and was not detected in October nor December (Table I-2). At no time did isolations from suspected cankers in the orchard where soil population studies were conducted indicate the presence of P. malorum.

Cankers did not develop where P. malorum spore suspensions were held in contact with uninjured bark (Table I-3). In the inoculations using plastic tubes which were made from December 1986 through June 1987, however, the bark was inadvertently injured by pressure placed on the tubes

Figs. I-4 a,b,c. Growth of cankers on Bosc pear trees induced by inoculation of mycelium of Phialophora malorum into wounded bark. Disks of bark 10 cm in diameter were removed and replaced with disks of mycelium of P. malorum grown on PDA. Control treatments received disks of sterile agar. Net length was determined by subtracting mean length of control cankers from mean length of cankers at inoculation sites.

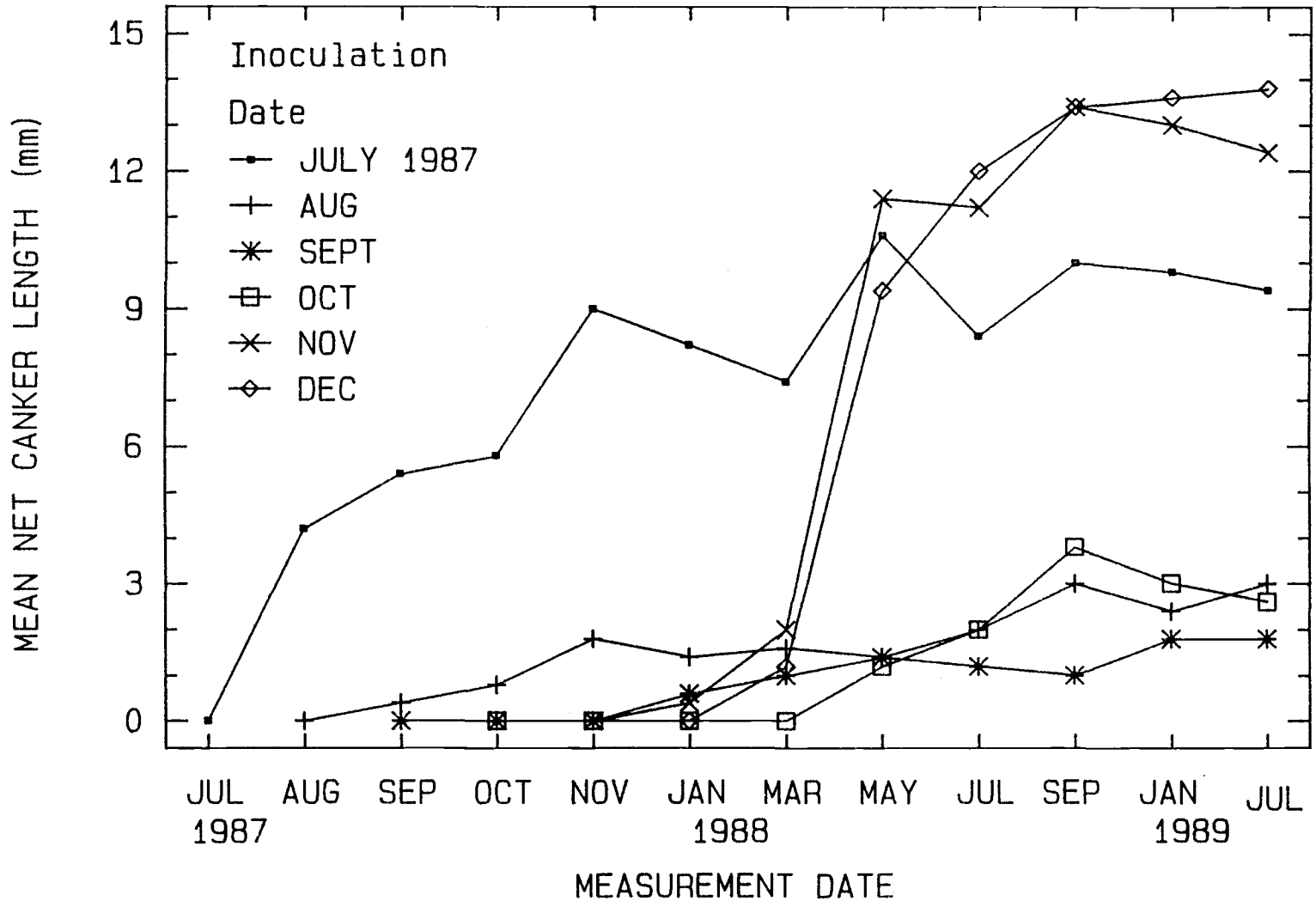
Inoculation
Date
JULY 1987
AUG
SEPT
OCT
NOV
DEC
MEAN NET CANKER LENGTH (mm)
0
3
6
9
12
15
JUL 1987
AUG
SEP
OCT
NOV
JAN 1988
MAR
MAY
JUL
SEP
JAN 1989
JUL
MEASUREMENT DATE

Fig. I-4a.

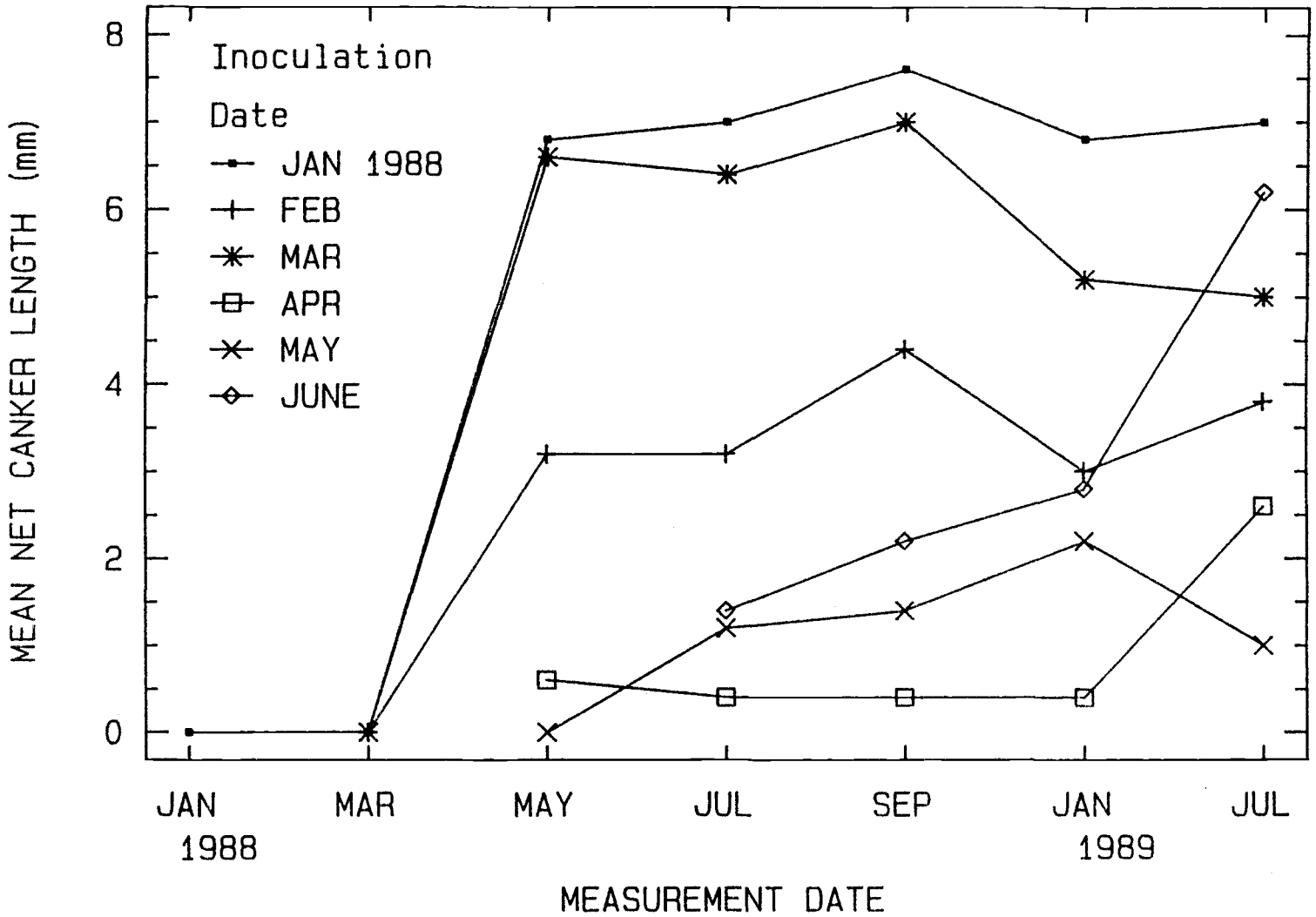
Inoculation
Date
JAN 1988
FEB
MAR
APR
MAY
JUNE
MEAN NET CANKER LENGTH (mm)
0
2
4
6
8
JAN
1988
MAR
MAY
JUL
SEP
JAN
1989
JUL
MEASUREMENT DATE

Fig. I-4b.

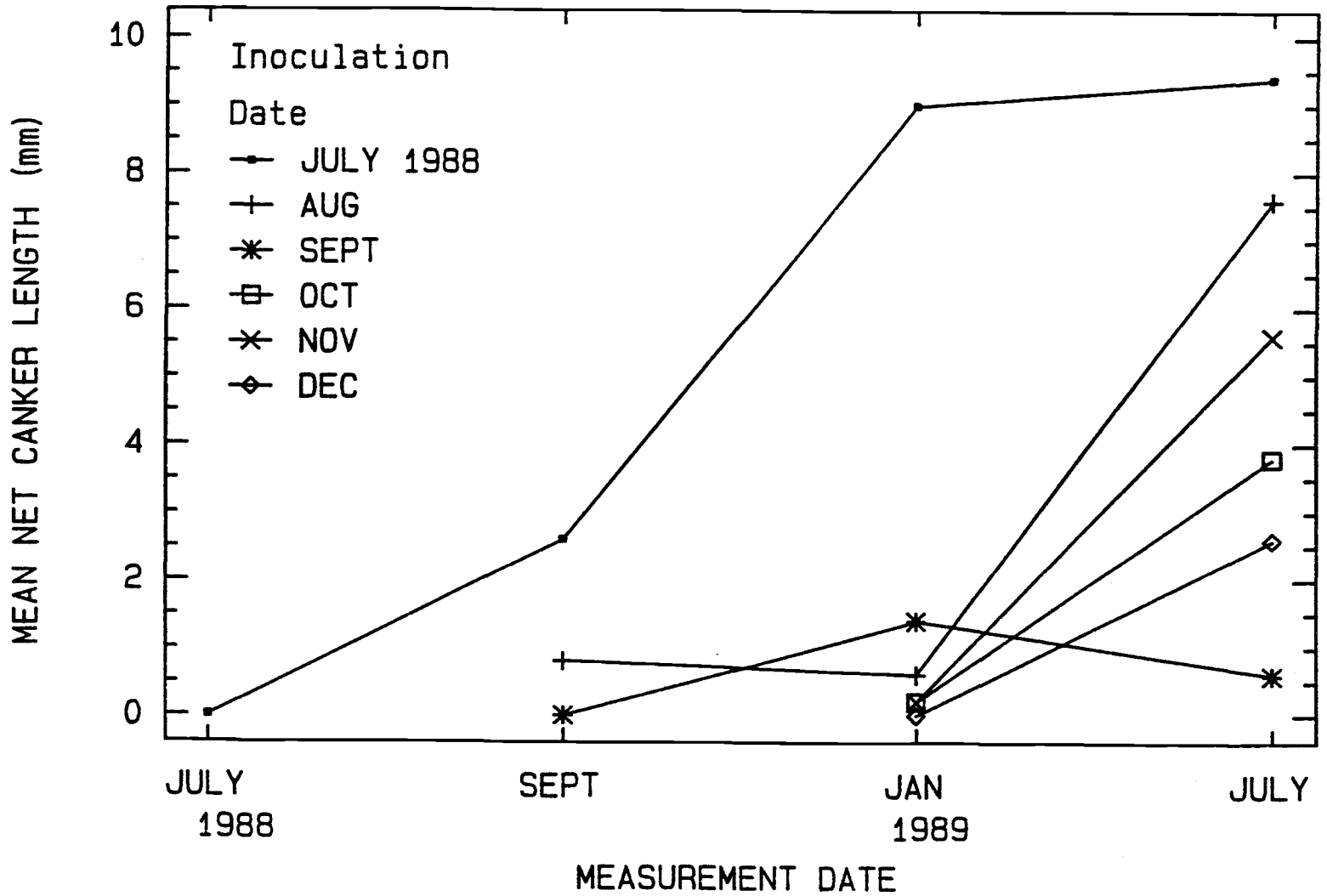
Inoculation
Date
JULY 1988
AUG
SEPT
OCT
NOV
DEC
MEAN NET CANKER LENGTH (mm)
10
8
6
4
2
0
JULY
1988
SEPT
JAN
1989
JULY
MEASUREMENT DATE

Fig. I-4c.

Table I-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 | | | | |
|---|---|---|---|---|---|
| | 4/88 | 6/88 | 8/88 | 10/88 | 12/88 |
| 1987 Sept | + | + | - | - | - |
| Sept | + | + | - | - | - |
| Nov | + | + | + | - | - |
| Nov | + | - | - | - | - |
| Dec | + | + | + | - | - |
| Dec | + | + | + | - | - |
| 1988 Feb | + | + | + | - | - |
| Feb | + | + | - | - | - |
| Mar | + | + | + | - | - |
| Mar | + | + | + | - | - |

Table I-3. Canker development on Bosc pear trees inoculated with spore suspensions of Phialophora malorum.

| Bark Treatment | Date | Number of cankers developing / 5 replicates | | | | | |
|---|---|---|---|---|---|---|---|
| | | Age of wood | | | | | |
| | | 1 yr | | 2 yr | | 3 yr | |
| | | Inoc. | Water | Inoc. | Water | Inoc. | Water |
| Wounded | 12/86 | 1 | - | 3 | - | 2 | - |
| | 1/87 | 4 | - | 5 | - | 4 | - |
| | 2/87 | 5 | - | 4 | - | 4 | - |
| | 3/87 | 0 | - | 0 | - | 0 | - |
| | 4/87 | 2 | - | 1 | - | 2 | - |
| | 5/87 | 4 | - | 3 | - | 4 | - |
| | 6/87 | 2 | - | 3 | - | 4 | - |
| Unwounded | 7/87 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 8/87 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 9/87 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 10/87 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 11/87 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 12/87 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1/88 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2/88 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 3/88 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 4/88 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 5/88 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 6/88 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 7/88 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 8/88 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 9/88 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 10/88 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 11/88 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 12/88 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1/89 | 0 | 0 | 0 | 0 | 0 | 0 |

during inoculation, and cankers frequently developed at these wound sites (Table I-3). P. malorum was consistently recovered in washes of cankers which developed following mycelial inoculation (Table I-4).

**Epiphytic populations.** P. malorum was recovered for more than 1 yr in washes from sites inoculated by spore suspension where cankers did not develop (Table I-5). The number of propagules in washes made in September declined significantly ($P=0.003$) as time from inoculation increased. However, this trend was not significant in washes made during late fall and early winter. P. malorum was not recovered from washes of control (distilled water) sites.

## DISCUSSION

The recovery of P. malorum from orchard soil over a 2-yr period indicates that the fungus is resident in soil, and that the soil may serve as an inoculum reservoir. This is further supported by the gradual rate of decline of propagule numbers in artificially infested soil in pots over 9 mo. Where fruit was added to infested soil, propagule levels did not rise until the fruit was 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 suggest that P. malorum utilizes nutrients released into the soil by other

Table I-4. Number of propagules of Phialophora malorum recovered in washes of cankers on Bosc pear trees.

| Sample date | Mean number of colonies/0.5 ml wash | SD[a] |
|---|---|---|
| 1988 28 June | 43.0 | 31.6 |
| 26 July | 19.2 | 8.3 |
| 18 Aug | 28.6 | 39.0 |
| 26 Aug | 46.4 | 42.8 |
| 08 Sept | 37.4 | 39.5 |
| 23 Sept | 55.2 | 35.5 |
| 07 Oct | 153.2 | 43.2 |
| 20 Oct | 110.8 | 41.8 |
| 15 Nov | 47.4 | 30.1 |
| 29 Nov | 215.6 | 108.3 |
| 15 Dec | 146.0 | 135.3 |
| 1989 04 Jan | 54.4 | 34.5 |
| 20 Jan | 60.4 | 39.2 |
| 31 Jan | 91.2 | 18.5 |
| 16 Feb | 86.0 | 45.3 |
| 15 Mar | 35.2 | 23.7 |
| 20 Apr | 28.4 | 35.4 |
| 17 May | 66.4 | 64.3 |
| 14 June | 19.4 | 14.6 |
| 11 July | 40.4 | 19.7 |

[a] SD = standard deviation.

Table I-5. Number of propagules of Phialophora malorum recovered from 3-yr old bark of Bosc pear trees following artificial infestation with spores of P. malorum.

| | Mean number of colonies/0.5 ml bark wash[b] | | | |
|---|---|---|---|---|
| | Sample date | | | |
| Infestation date[a] | 9/22/88 | 10/25/88 | 11/23/88 | 12/22/88 |
| 9/87 | 9.2 | 41.2 | 114.0 | 51.8 |
| 10/87 | 0.0 | 12.8 | 46.4 | 21.2 |
| 11/87 | 8.4 | 42.2 | 94.2 | 85.8 |
| 12/87 | 7.8 | 39.0 | 86.0 | 14.2 |
| 1/88 | 16.4 | 85.6 | 73.2 | 56.6 |
| 2/88 | 4.3 | 36.4 | 13.0 | 7.0 |
| 3/88 | 9.8 | 59.2 | 119.2 | 50.2 |
| 4/88 | 20.5 | 174.8 | 126.0 | 86.4 |
| 5/88 | 21.0 | 117.6 | 147.6 | 52.8 |
| 6/88 | 34.7 | 95.2 | 130.0 | 150.4 |
| 7/88 | 183.6 | 64.5 | 41.7 | 4.0 |
| 8/88 | 232.3 | 95.0 | 68.8 | 38.0 |
| 9/88 | 400.0 | 266.0 | 177.5 | 224.1 |
| Linear regression $p$ ($b \neq 0$): | .003 | .009 | .306 | .135 |

[a] Bark was infested by placing 0.25 ml of a suspension containing $10^5$ conidia/ml in plastic tubes secured to the bark surface and covered with parafilm.

[b] No P. malorum colonies were recovered from bark treated with distilled water at any infestation date.

microorganisms breaking down tissue of fallen pear fruit, and probably other organic matter sources. This pattern contrasts with that of Mucor piriformis Fischer, also a postharvest pathogen of pear, shown by Dobson et al (15) to increase rapidly in the presence of pear fruit on the orchard floor, and to be a frequent primary colonizer of pear tissue. 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 (46) of the relative weakness of P. malorum as a competitor in colonization of pear tissue during cold storage.

Since the number of propagules of P. malorum in soil with pear fruit added were increasing at the conclusion of the experiment (Fig. I-3), it is not clear how long relatively high propagule levels would be sustained by nutrients released from decayed fruit, or when a peak level would be reached. Under orchard conditions, the utilization by P. malorum of nutrients released from decaying fallen fruit is likely to be influenced by the particular soil flora present, environmental factors such as temperature and moisture, and the rate of contact between released nutrients and propagules of P. malorum. Although the distribution of propagules of P. malorum was not related to position relative to tree trunks, propagule distribution may be related to the positions of decayed fallen fruit on the orchard floor.

Successful induction of cankers in Bosc pear trees by inoculation with P. malorum was always associated with bark injury. While bark injuries may occur in orchards following events such as insect penetration, limb breakage, or pruning, the inability of P. malorum to infect through intact bark, taken together with the relatively brief longevity of the fungus in induced cankers, make it appear 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 relatively rapid period of canker development observed during March-May following inoculation during the previous summer or fall (Fig. I-4) 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 (20) reported colony growth of P. malorum on PDA at 12-34 C.

While P. malorum was recovered in washes of induced cankers, recovery in washes did not generally diminish after P. malorum was no longer detected in isolations from cankered tissue, indicating that epiphytic populations may have been a more critical source of propagules than sporulation from cankers per se. Recovery of P. malorum in washes of non-cankered bark sites up to 1 yr after inoculation by spore suspension indicates that epiphytic

survival is possible, and epiphytic populations may also serve as sources of inoculum. However, non-recovery at control sites on neighboring branches over the same period indicates that such epiphytic colonization may not be widespread. In addition, related experiments (Chapter 3) showed that fruit were not infested with P. malorum during the growing season, indicating that spores, if present on the bark, were not being transferred to the fruit.

# Chapter II.

## Effects of soil temperature, pH, and matric potential on population dynamics of *Phialophora malorum*.

## INTRODUCTION

Side rot of pear, caused by *Phialophora malorum* (Kidd & Beaum.) McColloch, is an important disease of stored pears in the Rogue River Valley of southern Oregon (6,7,43). Side rot is a disease of long-term stored fruit, rarely observed before 3 mo storage, and appearing more frequently after pears have been stored 4-5 mo at -1 C (43). *P. malorum* has been found to be resident in orchard soil (Chapter 1), which may be an important inoculum reservoir for postharvest fruit decay.

Few studies have reported on the population responses of postharvest pathogens to changes in soil conditions. Michailides and Ogawa (32), in their study of *Mucor piriformis* Fischer, also a postharvest pathogen of pear, demonstrated that sporangiospores germinated poorly at temperatures > 27 C, and this effect was more pronounced in wet (-0.3 bar matric potential) than in dry (-1300 bar) soil. However, although survival of sporangiospores and

mycelia of M. piriformis was reduced at soil temperatures >27 C, survival was greater in drier than in wetter soil.

Spotts (38) observed that although populations of P. malorum on the surface of Anjou pear fruit declined over time, the conidial survival half-life ($T_{50}$) was greater at 30 C than at 20 C. Gardner (20) reported colony expansion of P. malorum on potato dextrose agar (PDA) at all temperatures tested from 12-34 C, with most rapid growth at 23-27 C. Spores germinated in tap water over the same range of temperatures and at pH values from 3.4 - 8.0, with most vigorous germination observed at pH 4.4 - 5.8. McColloch (30) also observed most rapid colony development in culture at pH 4.4 - 6.0.

The objective of this study was to determine the response of spores of P. malorum to various levels of soil temperature, pH, and matric potential, in order to understand characteristics of the fungus which may be useful in explaining patterns of disease occurrence and in the development of control stategies.

## MATERIALS AND METHODS

All studies of soil populations of P. malorum were conducted using non-sterile soil from a mature pear orchard where P. malorum had been found previously (Chapter 1). The soil is classified as Kerby Loam, 0-3 % slope (48), and the

pH of the top 1-3 cm of soil was 5.2. A moisture release curve relating volumetric water content to matric potential for unamended soil is shown in Fig. II-1. This relationship was determined by the Oregon State University Soil Physics Lab, Corvallis.

**Soil temperature.** The effects of soil temperature on populations of P. malorum were determined by incubation of artificially infested soil at 0, 5, 10, 15, 20, 25, and 30 C for 0, 1, 2, and 3 mo. Five g of air-dried soil was placed in each of four replicate 50 ml glass tubes per sampling date at each temperature and infested by addition of 1 ml of water containing $10^5$ conidia. The conidial suspension was prepared by washing 3-wk-old cultures of P. malorum growing on potato dextrose agar (PDA)(Difco) with distilled water and adjusting the concentration after counting conidia on a hemocytometer. Cultures of P. malorum were originally isolated from lesions in pear fruit. All tubes were covered with parafilm. Tubes of the 25 and 30 C treatments were placed in heat blocks in the laboratory, while the remainder were placed in controlled-environment chambers at the desired temperatures. The moisture content of the infested soil was determined by weighing the soil from four replicate tubes before and after drying to constant weight in an oven at 100 C.

At each sampling date, soil from four replicate tubes at each temperature was added to 95 ml distilled water,

Fig. II-1. Water release curve relating soil matric potential to volumetric water content of an orchard soil mixed 2:1 with coarse sand. Values determined by the Oregon State University Soil Physics Lab, Corvallis.

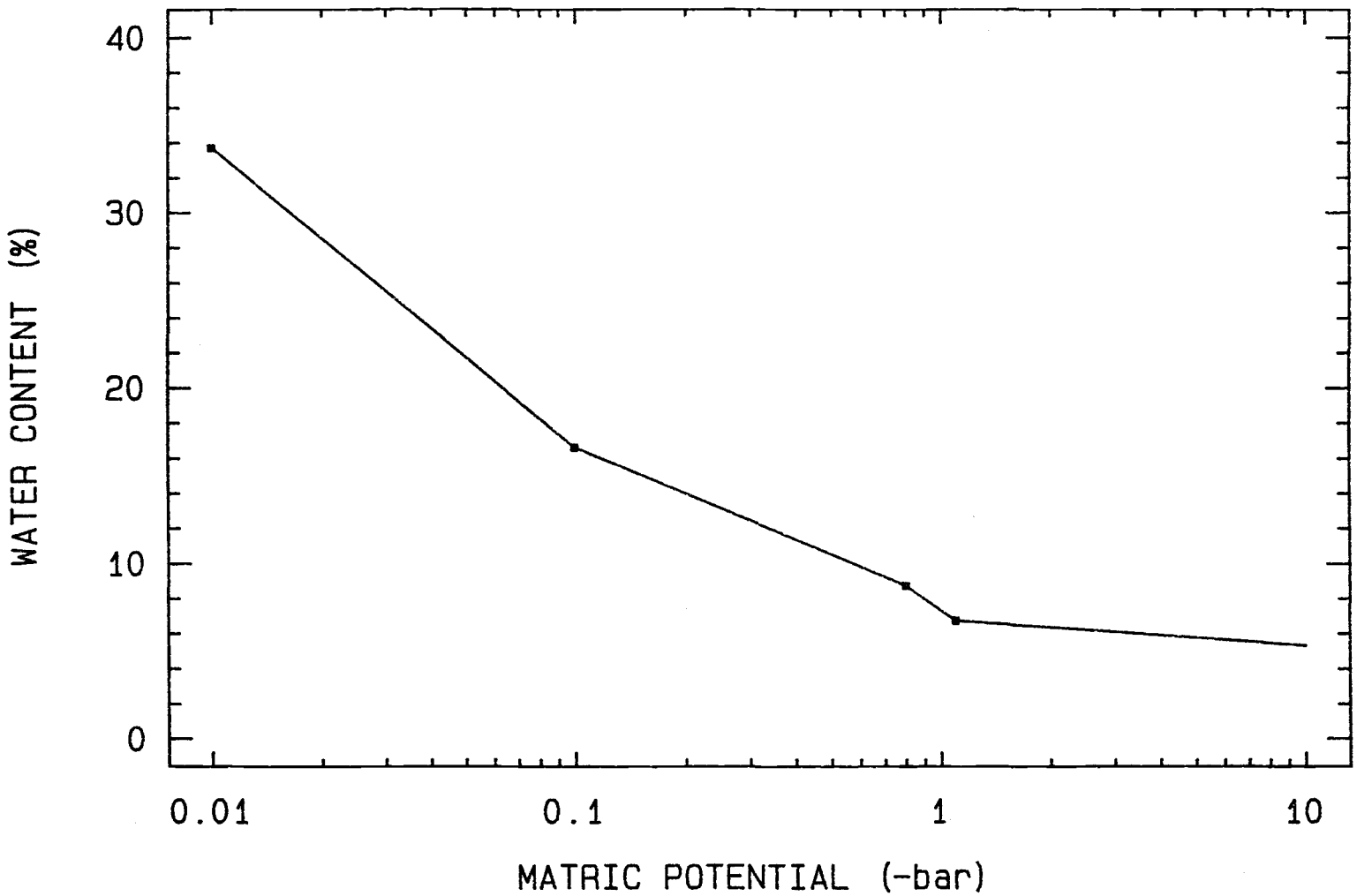


Fig. II-1.

agitated on a wrist-action shaker for 20 min, and diluted 1:100 in distilled water. 0.5 ml aliquots of the dilution were spread on each of five plates of a semi-selective medium (SSM) containing benomyl, DCNA, streptomycin sulfate, rose bengal, and PDA (Chapter 1). After 6-8 wk incubation at 20 C, colonies of P. malorum on each plate were counted and propagules per gram dry soil calculated. Colonies of P. malorum were identified by colony morphology and color, and microscopic examination of the shape and size of conidiogenous cells and conidia (30).

**Soil matric potential.** In matric potential studies, orchard soil mixed with coarse sand (2:1, soil:sand) was infested with spores of P. malorum to an initial inoculum concentration of approximately $10^5$/g dry soil. A moisture release curve relating volumetric water content to matric potential for this soil mixture was developed by the Oregon State University Soil Physics Lab and is shown in Fig. II-2. Infested soil was placed in 250 ml Buchner funnels with fritted glass plates as bottoms (VWR Scientific) and continuous columns of water were established in plastic tubes connecting ends of the funnels to water reservoirs. Constant distances were maintained between the fritted glass plates and the water level in the reservoirs. Based on the approximate equivalency 1 cm water column = -1 millibar (mb) matric potential (12,22,24), soil matric potential ($\psi_m$) values of 0, -10, -100, -300, and -1000 mb were established.

Fig. II-2. Water release curve relating soil matric potential to volumetric water content of an orchard soil. Values determined by the Oregon State University Soil Physics Lab, Corvallis.

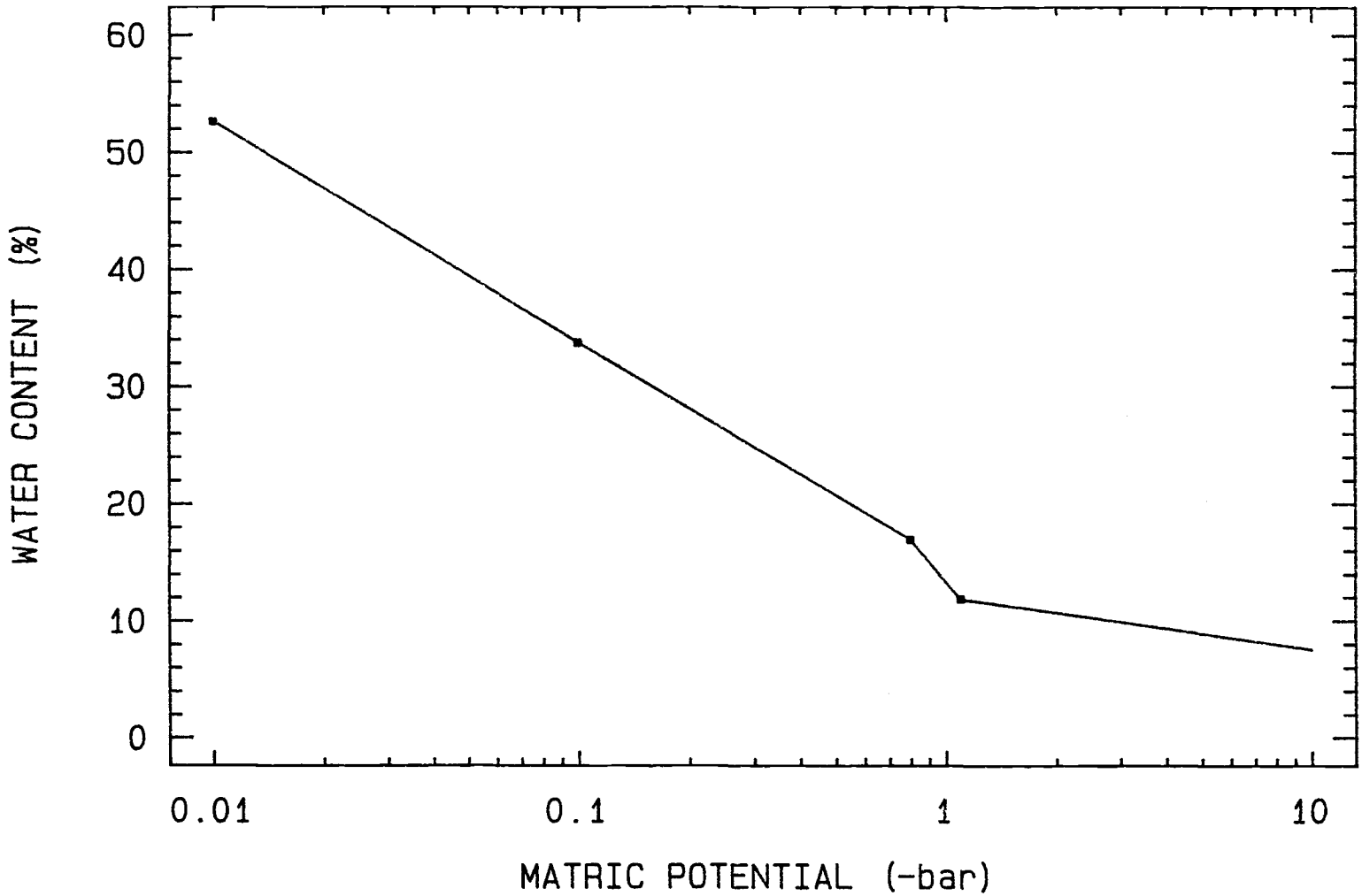
60
50
40
30
20
10
0
WATER CONTENT (%)
0.01
0.1
1
10
MATRIC POTENTIAL (-bar)

Fig. II-2.

A flooded soil treatment ( $\psi_m > 0$) was established by maintaining the reservoir level 1 cm higher than the soil level in the funnel. Each funnel was loosely covered with a plastic bag to reduce evaporation. Temperatures ranged from 20-25 C during the experiment. At monthly intervals over a 7 mo period, three replicate samples of approximately 5 g soil were removed from each funnel, and the number of propagules of P. malorum per gram dry soil were determined by dilution plating on SSM as described above.

**Soil pH.** The effect of soil pH on populations of P. malorum was determined by placing pH-amended, infested soil in Buchner funnels maintained at $\psi_m$ = -25 mb, according to the methods described in matric potential experiments above. Funnels were maintained 7 mo at 20 C. Soil pH values of 3, 4, 5, 6, and 7 were established by thoroughly mixing either 1N NaOH or concentrated HCl with soil until the desired values were obtained. The pH values were checked periodically and maintained at +/- 0.5 pH unit by further addition of NaOH or HCl when necessary. At monthly intervals over a 7-mo period, three replicate samples of approximately 5 g soil were removed from each funnel, and the number of propagules of P. malorum per gram dry soil were determined by dilution plating on SSM as described above.

# RESULTS

**Soil temperature.** P. malorum was recovered from soil at all temperatures tested over the 3-mo experiment (Fig. II-3). However, populations declined over time where soil temperatures were 10 C or higher, with the rate of decline in the number of propagules per gram dry soil generally increasing with temperature. At 0 and 5 C, the number of propagules per gram dry soil did not significantly decline over the course of the experiment.

**Soil matric potential.** In flooded soil, populations of P. malorum declined sharply and were not detected after incubation of 4 mo or longer (Fig. II-4). Populations declined to relatively low levels in $\psi_m$ = 0 and -10 mb treatments, and were not detected at 7 mo at $\psi_m$ = 0. The greatest overall survival of P. malorum in soil occurred at $\psi_m$ = -300 and $\psi_m$ = -1000 mb.

**Soil pH.** Populations of P. malorum declined over time at all pH-values tested (Fig. II-5). The rate of decline in the number of propagules per gram dry soil over the 7-mo experiment decreased with increasing pH-value. At pH 3 and pH 4, propagules were not detected after 7 mo. At pH 3, propagule levels dropped abruptly from the initial level to the 1-mo sample date. The initial drop in propagule levels was less abrupt as pH increased, and at pH 6 and pH 7

Fig. II-3. Effect of temperature on survival of Phialophora malorum in a non-sterile orchard soil. Five g soil was placed in each of four replicate glass tubes per sampling date at each temperature and infested by addition of 1 ml water containing $10^5$ conidia/ml. Number of propagules per gram dry soil was determined by dilution plating.

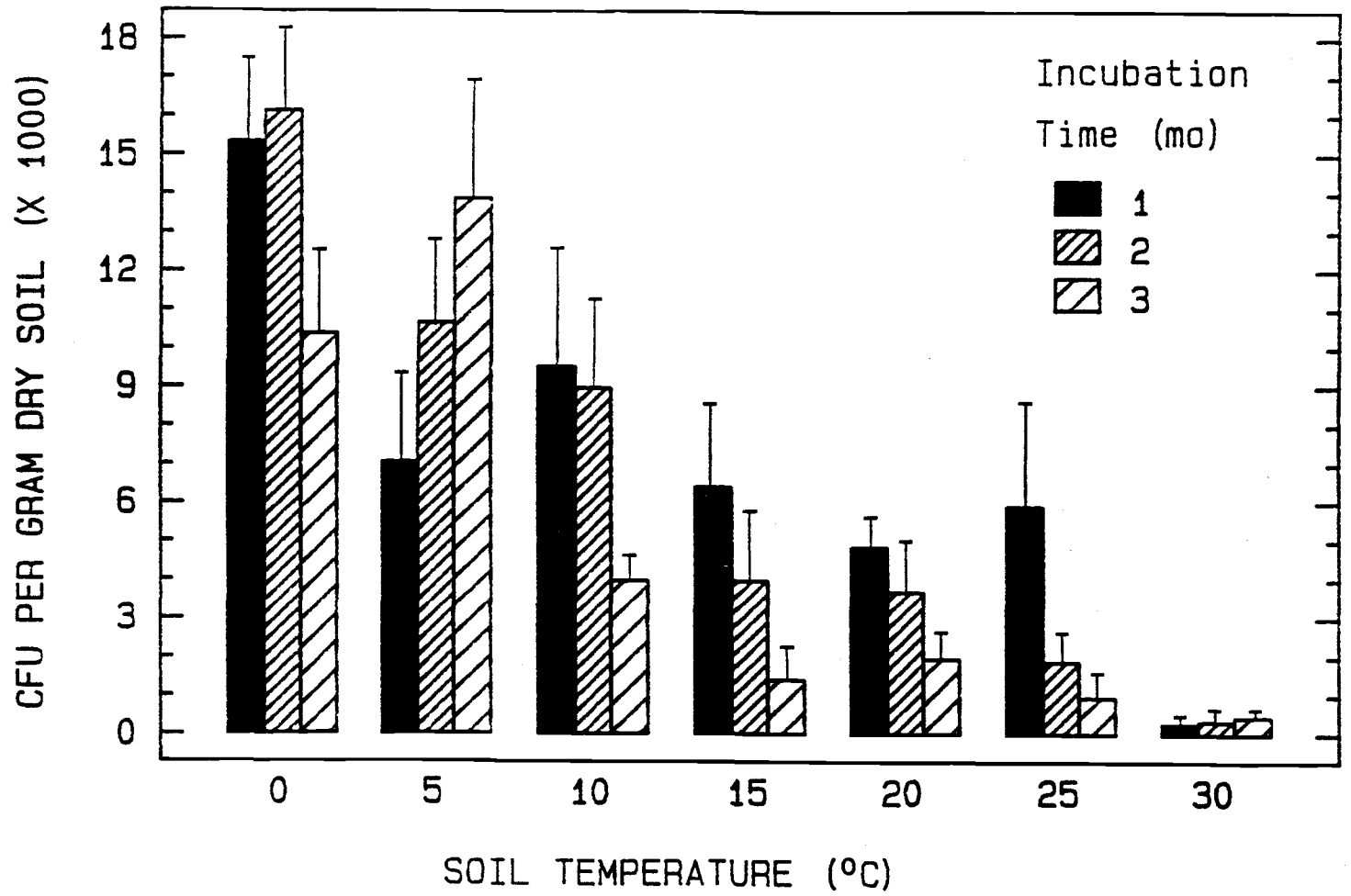
CFU PER GRAM DRY SOIL (X 1000)
18
15
12
9
6
3
0
Incubation
Time (mo)
1
2
3
0
5
10
15
20
25
30
SOIL TEMPERATURE (°C)

Fig. II-3.

Fig. II-4. Effect of soil matric potential on survival of Phialophora malorum in a non-sterile orchard soil. An orchard soil mixed 2:1 with sand was infested with conidia of P. malorum and placed in funnels with bottoms of fritted glass maintained at the desired matric potential values. Three replicate soil samples were taken from each funnel monthly and numbers of propagules per gram dry soil determined by dilution plating. Initial propagule level was $6.5 \times 10^4$ CFU/g dry soil.

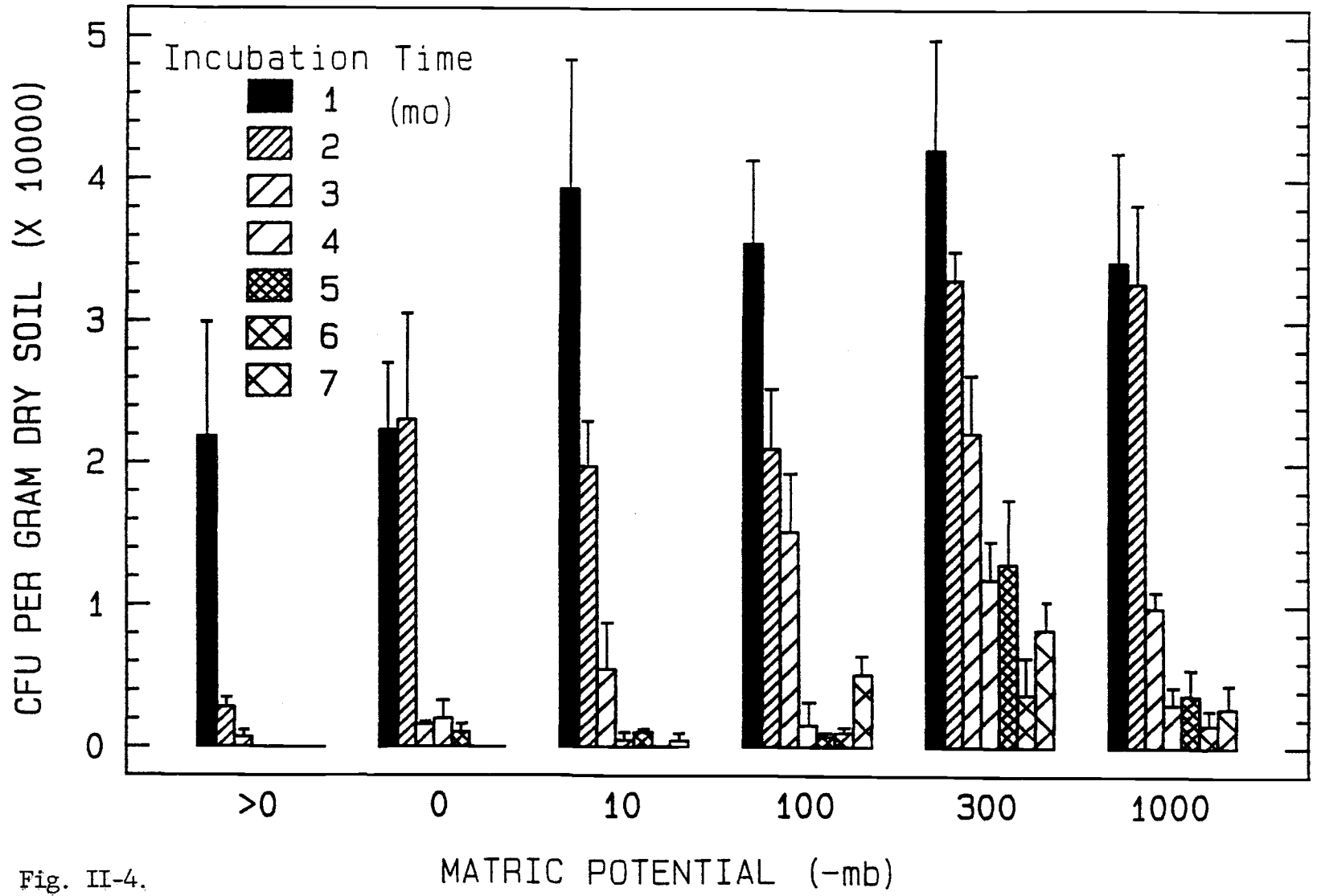
Incubation Time
(mo)
1
2
3
4
5
6
7
CFU PER GRAM DRY SOIL (X 10000)
0
1
2
3
4
5
>0
0
10
100
300
1000
MATRIC POTENTIAL (-mb)

Fig. II-4.

Fig. II-5. Effect of soil pH on survival of Phialophora malorum in a non-sterile orchard soil. Initial soil pH was 5.2, and was maintained at +/- 0.5 pH units indicated by addition of NaOH or HCl. Soil in all treatments was maintained at a matric potential of -25 mb at 20 C. Three replicate soil samples were taken monthly, and numbers of propagules per gram dry soil determined by dilution plating. Initial propagule levels were 1.2 - 1.3 X $10^5$/g dry soil.

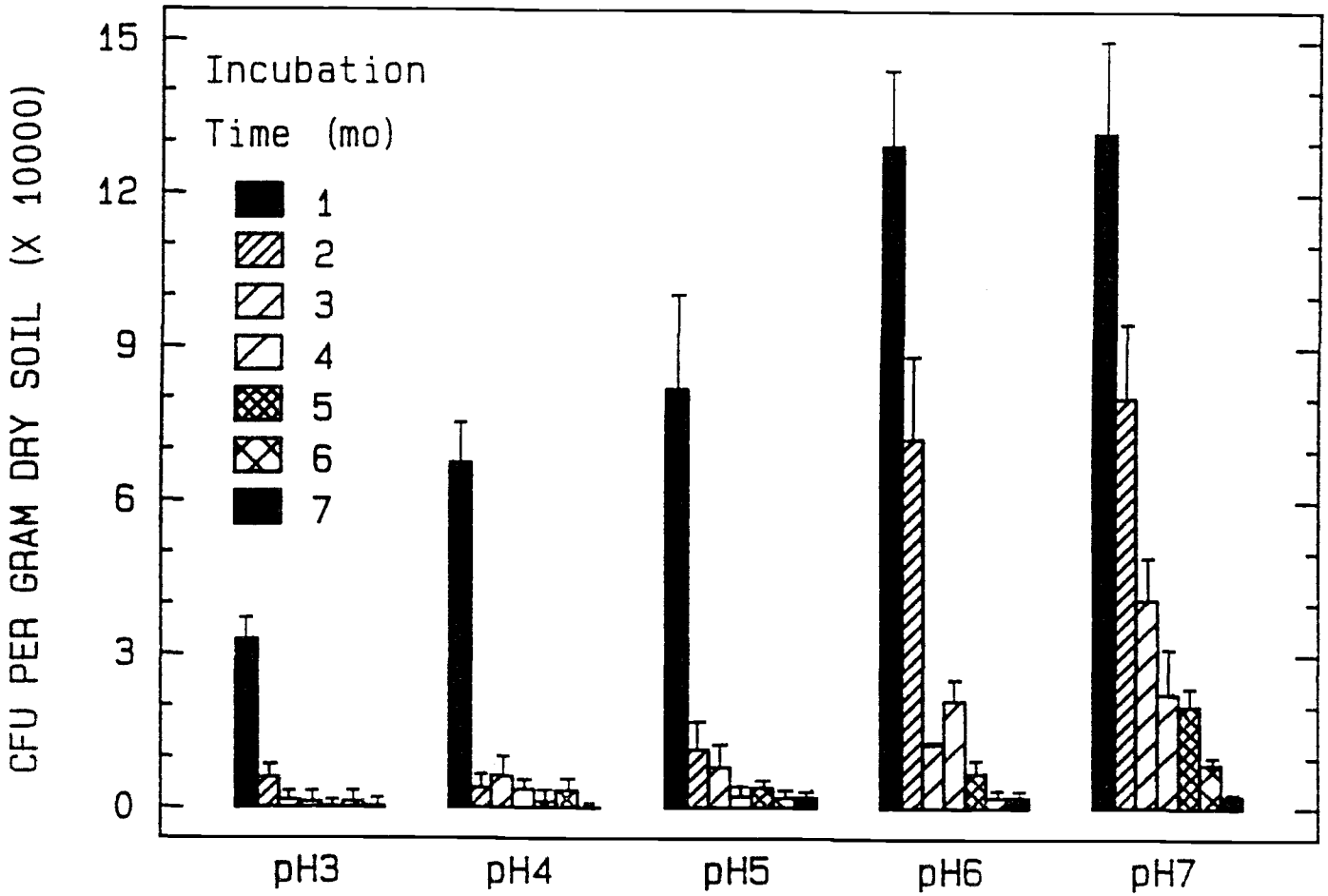
CFU PER GRAM DRY SOIL (X 10000)
15
12
9
6
3
0
Incubation
Time (mo)
1
2
3
4
5
6
7
pH3
pH4
pH5
pH6
pH7

Fig. II-5.

propagules per gram dry soil were unchanged after 1 mo as compared to inital levels.

## DISCUSSION

Based on the three soil environmental factors tested in this study, P. malorum is capable of survival under a broad range of soil conditions and over an extensive time period without input of exogenous nutrients. It is not unlike many soil microorganisms in having survival enhanced by a cool, dry soil environment, in which activity of competing and antagonistic microorganisms may be suppressed (21,32). Populations of P. malorum were reduced to relatively low levels by low soil pH (Fig. II-5), very wet or flooded soil (Fig. II-4), and high soil temperatures (Fig. II-3). However, the ability of P. malorum to endure these conditions is particularly relevant to the disease cycle of the fungus, since soil survival is critical to the continuity of inoculum, and populations may increase rapidly with input of nutrients, as shown by the response to decayed pear fruit in the soil (Chapter 1). Although P. malorum was not detected in flooded soil after 3 mo of incubation, flooded conditions are not likely to persist in commercial pear orchards for such extensive periods. Several orchards in southern Oregon with known histories of side rot problems are located on well-drained soils (D. Sugar, personal

observation). This may reflect the relatively poor survival of P. malorum under wet conditions.

The survival of P. malorum at high soil temperatures is critical to its ability to over-summer in southern Oregon, where maximum soil temperatures at 5 cm depth occasionally exceed 30 C (Fig. II-6). While populations declined at high temperatures, P. malorum appears to be less sensitive to high soil temperature than Mucor piriformis Fischer, also a soil-borne postharvest pathogen of pear, which declined in population rapidly at 27 C, and few sporangiospores were viable after incubation at 33 C (32). This may explain why M. piriformis is rarely isolated as a causal agent of postharvest pear decay in southern Oregon (D. Sugar, personal observation), where P. malorum is a problem, while M. piriformis is a frequent problem in the Mid-Columbia pear growing district of northern Oregon, where temperatures are generally cooler. P. malorum has not been reported from rotting pear fruit nor from soil in the Mid-Columbia district (R.A. Spotts, personal observation). Wetter conditions in the Mid-Columbia district may favor suppression of P. malorum, although the fungus may simply not be present in that geographical area. Wong (49) studied survival in soil of two other species in the form-genus Phialophora, and also found greatest survival under cool dry conditions, while these fungi were virtually eliminated from warm, moist soil.

Fig. II-6. Soil temperature at Medford, Oregon at 5 cm depth from January 1987 to August 1989. Data from National Weather Service, Medford.

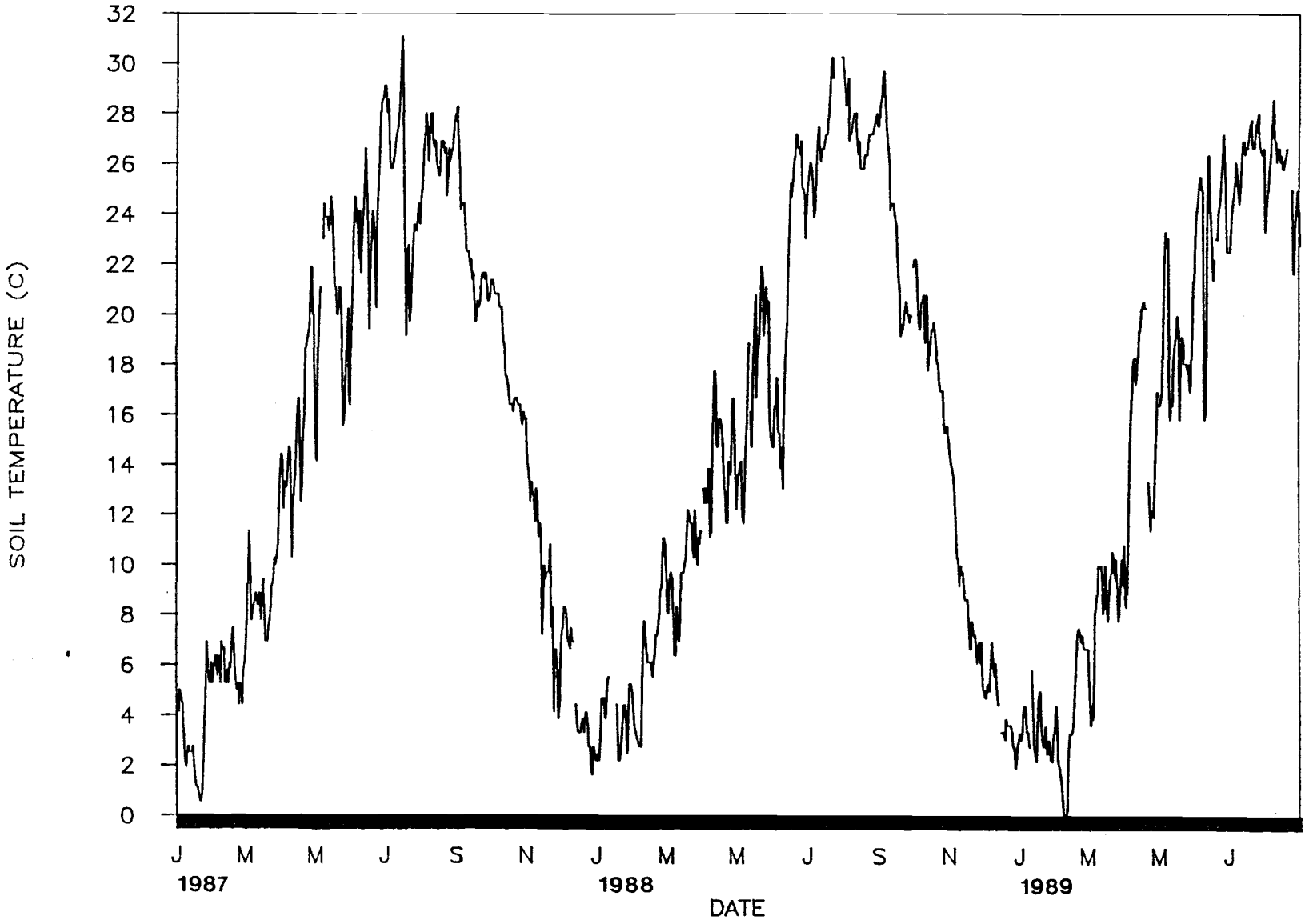
SOIL TEMPERATURE (C)
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
J M M J S N J M M J S N J M M J
1987
1988
1989
DATE

Fig. II-6.

Gardner (20) reported that P. malorum sporulated on PDA at 12-34 C, and that optimum mycelial growth occurred between 23-27 C, indicating a tolerance for relatively high temperatures in culture. He also indicated that chlamydospores were produced in culture at 30 and 34 C. The type of survival structures involved in survival in soil in this study were not determined.

The contrast between the growth of P. malorum at relatively high temperatures in culture and the rapid decline of populations at high soil temperatures may reflect both a response to nutrient depletion and the sensitivity of P. malorum to competing microorganisms. P. malorum was not found decaying pear fruit in inoculated soil or orchard soil (Chapter 1), and its infection of pear fruit may be inhibited when wounds are colonized by other pathogens (46). Thus P. malorum can be appropriately described as a weak pathogen of pear, and this lack of aggressiveness apparently applies to soil interactions as well.

# Chapter III.

## Dispersal of inoculum of *Phialophora malorum* from orchard soil and inoculum redistribution in pear immersion tanks.

## INTRODUCTION

Side rot of pear, caused by *Phialophora malorum* (Kidd & Beaum.) McColloch, is an important disease of stored pears in the Rogue River Valley of southern Oregon (6,7,43). *P. malorum* has been found to be resident in orchard soil (Chapter 1), 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 (30) 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 (31) found *Mucor piriformis* Fischer at significant propagule levels in orchard soil and debris, decaying fallen fruit on the orchard floor, in soil adhering to harvest bins, and in packinghouse immersion dump tanks, but not in 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 (8,9,10,11,25,41). Botrytis cinerea Pers.: Fr., Penicillium expansum Link ex Thom, and Cladosporium herbarum (Pers.) Link ex Gray have been associated with decaying plant debris in soil (1,3,18,34) and were found by Michailides and Spotts in orchard air and in washings of pear fruit while on the tree (31). They also recovered Alternaria alternata (Fr.) Keissler from orchard air.

The objective of this study was to determine how inoculum of P. malorum is dispersed from orchard soil, and when pear fruit become infested.

## MATERIALS AND METHODS

**Orchard studies.** Studies were conducted in a commercial orchard with a history of side rot incidence to determine if pears become infested while on the tree or during harvest operations. To sample orchard air, 10 plates of PDA were exposed for 5 min at random sites throughout a 0.5-ha test area at approximately biweekly intervals for 18 mo. Plates were evaluated for development of P. malorum after 2-4 wk incubation at 20 C. To determine fruit infestation, 20 fruit/tree on five randomly chosen trees were washed biweekly during the 1987 and 1988 growing seasons. Approximately 2 ml of distilled water was sprayed onto fruit

surfaces with a hand-operated atomizer and runoff water was collected in a plastic jar. Runoff water from 20 fruit/tree was pooled. In the laboratory, runoff water from each tree was centrifuged 10 min at 2000 rpm to concentrate spores, and 0.5 ml withdrawn from the bottom of each centrifuge tube and plated on a semi-selective medium (SSM) prepared as described previously (Chapter 1). At harvest, washes were made of 20 fruit/bin from each of 5 randomly chosen harvest bins in the orchard test area.

In an additional study of the timing of fruit infestation, 10 fruit/tree on five randomly selected trees were covered with paper bags, closed around the fruit spur with plastic tape to prevent deposition of spores. Fifty fruit were bagged biweekly during the 1987 and 1988 growing seasons, and remained bagged through harvest. Fruit were kept in bags during harvest. After cooling to 0 C, fruit from each bagging date from each replicate tree were individually treated with benomyl (300 mg/L) as a line-spray to reduce competition from fungi other than P. malorum, which is not sensitive to benomyl (7,45). Fruit were stored in polyethylene-lined boxes for 5 mo at 0 C, when decay was evaluated and causal fungi identified. Ten non-bagged fruit were harvested from each tree as controls.

To further evaluate whether or not fruit were latently infected prior to harvest, fruit from the test orchard were sampled during commercial packing. Ten boxes of 100 fruit

each were collected at 20 min intervals from packing lines after fruit had passed through the immersion dump tank. The fruit were then surface-sterilized by 5 min immersion in water containing 0.5 % sodium hypochlorite, and stored for 5 mo at -1 C in polyethylene-lined cardboard cartons. Incidence of side rot in the fruit was compared to that in 10 commercially packed boxes randomly selected during the same time period, which did not receive sodium hypochlorite dips. Identity of causal fungi was confirmed by isolation on PDA from lesion margins. P. malorum was identified by colony morphology and color, and microscopic examination of the shape and size of conidiogenous cells and conidia (30). Treatment means were compared by Student's t test (two-tailed) for unpaired data, using Number Cruncher Statistical System software (J.L. Hintze, Kaysville UT).

**Packinghouse studies.** 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 to reduce populations of pathogenic fungi (16,26,40,47). To determine if pear fruit may become infested with P. malorum during the packing process, samples were taken of packinghouse air and from dump tank solutions before and after pear dumping and the number of propagules of P. malorum was determined. Samples were taken during commercial packing of Bosc pears in 1985. In the cooperating packinghouse, immersion tanks were

emptied, cleaned, and refilled prior to packing on the first and fourth day of each week. Fresh tank solutions were sampled prior to pear dumping on the first day of each week for 3 weeks, 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 distilled water. 0.5 ml of the dilution was spread on each of five plates of potato dextrose agar (PDA)(Difco) and colonies which developed were counted after 2-4 wk incubation at 20 C. At each sample date, packinghouse air was sampled by exposing 4 plates of PDA for 5 min at various locations in the packinghouse.

**Inoculum redistribution in immersion tanks.** Since immersion dump tanks are known to harbor spores of pathogenic fungi (8,10,11,25,41), experiments were designed to evaluate the potential for exchange of spores of P. malorum between fruit surfaces and the dump tank solution. To measure the transfer of spores from fruit to the tank solution, the entire 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 2-4 wk old colonies growing on PDA with distilled water. The spore concentration was adjusted to $10^4$/ml using a hemacytometer. After air-drying on a laminar-flow sterile bench, five of the fruit were peeled, peels were weighed,

and blended in 20 ml distilled water for 5 min in a Waring Blendor at high speed. Five 0.5-ml aliquots of the blended suspension were plated on PDA, and colonies of P. malorum were counted after 2-4 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 tap water at 10 C, and peels were removed, blended, and plated as above after air drying. The water was changed and the tank cleaned with 95% ethanol in water after each fruit immersion. Five 0.5-ml aliquots were removed from the water before and after immersion of each fruit, and plated on PDA as above.

In a separate experiment, five Bosc pears were surface-sterilized for 5 min in 0.5% sodium hypochlorite, rinsed in fresh water, air dried, and then individually immersed 2 min in the circulating water bath containing $10^4$ spores of P. malorum per ml. The water was changed and tank cleaned following immersion of each fruit. The number of spores carried from the bath on fruit surfaces was measured by peeling, blending, and plating on PDA as above.

## RESULTS

**Packinghouse studies.** P. malorum was not recovered from packinghouse air at any sampling date, nor was it recovered

from immersion tank solutions prior to dumping of pears. However, P. malorum was recovered from tank solutions after bins of pears had been immersed at all sampling dates but one. The mean concentration of propagules of P. malorum in the tank solutions ranged from 0-352/ml (Table III-1).

**Orchard studies.** P. malorum was not recovered from orchard air during the growing season, but the fungus was detected in air samples collected during harvest in 1987 (Table III-2). At that sample date, P. malorum colonies developed on five of the 10 sample plates, a total of seven colonies developing. P. malorum was not detected in air during the 1988 harvest season. P. malorum was not recovered from fruit surfaces during the growing season (Table III-3), but 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 (Table III-4), nor in fruit from the same trees that were not bagged.

No side rot developed during storage in fruit immersed in sodium hypochlorite solution, while side rot occurred in an average of 1.1 % of the commercially packed fruit (Table III-5).

**Inoculum redistribution in immersion tanks.** Immersion of artificially infested pears for 2 min in a circulating water bath resulted in an average of 41% of the spores of P. malorum being transferred from the fruit surface to the tank

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

| Sample date | Packinghouse air 5 min exposure[a] (colonies/plate) | Immersion tank before dumping[b] (spores/ml) | Immersion tank after dumping[c] (spores/ml +/-SD) |
|---|---|---|---|
| 10/21/85 | 0 | 0 | 48.0 +/- 33.5 |
| 10/22/85 | 0 | - | 320.0 +/- 93.8 |
| 10/23/85 | 0 | - | 112.0 +/- 52.2 |
| 10/28/85 | 0 | 0 | 104.0 +/- 82.9 |
| 10/29/85 | 0 | - | 0.0 |
| 10/30/85 | 0 | - | 230.0 +/- 75.7 |
| 11/04/85 | 0 | 0 | 352.0 +/- 76.9 |
| 11/05/85 | 0 | - | 120.0 +/- 40.0 |
| 11/06/85 | 0 | - | 216.0 +/- 35.8 |

[a] At each sample date, 4 plates of potato dextrose agar were placed in various locations in the packinghouse and lids removed for 5 min.

[b] On the first packing day of each week, tanks were drained, cleaned, and filled with fresh water, sodium carbonate to S.G. 1.04, and sodium *o*-phenyl phenate (0.3%). Samples were tanken before dumping of pears.

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

Table III-2. Recovery of *Phialophora malorum* from air in a commercial orchard.

| Sample date | Colonies of *P. malorum* developing following 5 minute exposure in orchard | |
|---|---|---|
| | Mean of 10 plates | No. plates with colonies |
| 07/21/87 | 0 | 0 |
| 08/04/87 | 0 | 0 |
| 08/20/87 | 0 | 0 |
| 09/01/87 | 0.7 | 5 |
| 09/15/87 | 0 | 0 |
| 10/01/87 | 0 | 0 |
| 10/27/87 | 0 | 0 |
| 11/28/87 | 0 | 0 |
| 12/19/87 | 0 | 0 |
| 01/06/88 | 0 | 0 |
| 02/12/88 | 0 | 0 |
| 03/09/88 | 0 | 0 |
| 04/01/88 | 0 | 0 |
| 05/07/88 | 0 | 0 |
| 06/23/88 | 0 | 0 |
| 07/07/88 | 0 | 0 |
| 07/21/88 | 0 | 0 |
| 08/04/88 | 0 | 0 |
| 08/17/88 | 0 | 0 |
| 08/31/88 | 0 | 0 |
| 09/08/88 | 0 | 0 |
| 09/15/88 | 0 | 0 |
| 09/29/88 | 0 | 0 |
| 10/13/88 | 0 | 0 |
| 10/27/88 | 0 | 0 |
| 11/15/88 | 0 | 0 |
| 12/16/88 | 0 | 0 |
| 01/26/89 | 0 | 0 |
| 02/09/89 | 0 | 0 |

Table III-3. Recovery of *Phialophora malorum* from fruit surfaces in a commercial orchard.

| Sample date | Colonies of P. malorum developing from 20 fruit washed per tree: Mean of 10 trees | No. trees yielding colonies |
|---|---|---|
| 07/21/87 | 0 | 0 |
| 08/04/87 | 0 | 0 |
| 08/20/87 | 0 | 0 |
| 09/01/87[a] | 0.92 | 7 |
| 06/23/88 | 0 | 0 |
| 07/07/88 | 0 | 0 |
| 07/21/88 | 0 | 0 |
| 08/04/88 | 0 | 0 |
| 08/17/88 | 0 | 0 |
| 08/31/88[a] | 0 | 0 |

[a] At harvest, 20 fruit were washed from each of 10 harvest bins selected randomly through the experimental block.

Table III-4. Incidence of side rot in Bosc pears covered with paper bags at various times during the growing season.

| Date fruit covered[a] | Side rot incidence | Harvest date |
|---|---|---|
| 1986 | | |
| 28 July | 0 | |
| 04 August | 0 | |
| 11 August | 0 | |
| 18 August | 0 | |
| 25 August | 0 | |
| Not covered | 0 | |
| | | 01 September |
| 1987 | | |
| 07 August | 0 | |
| 14 August | 0 | |
| 22 August | 0 | |
| 28 August | 0 | |
| Not covered | 0 | |
| | | 03 September |

[a] Fruit remained covered through harvest.

Table III-5. Evaluation of latent infection in Bosc pears from an orchard with *Phialophora malorum* in the soil.

| Treatment[a] | Mean % side rot[b] |
|---|---|
| Surface-sterilized | 0.00 |
| Commercially packed (not surface-sterilized) | 1.10 |

[a] Ten randomly selected 100-fruit commercially packed boxes were compared to 10 boxes removed from packing lines and immersed 5 min in 0.5 % sodium hypochlorite.

[b] Treatment means are significantly different ($P < 0.01$) according to Student's $t$ test (non-paired).

water (Table III-6). Surface-sterilized fruit immersed for 2 min in the circulating bath containing $10^4$ spores of P. malorum per ml carried an average of 764.9 spores per g peel out of the water.

## DISCUSSION

Regular recovery of P. malorum from immersion tank solutions during pear dumping, while the fungus was detected neither in packinghouse air nor in fresh tank solutions, indicates that propagules of P. malorum are carried into the packinghouse on fruit and/or on harvest bins. Since P. malorum is known to be resident in orchard soil (Chapter 1), the fungus clearly could be carried in soil adhering to harvest bins or otherwise entering bins during harvest. Bins are moved about in the orchard by tractor-mounted forklifts, and often become contaminated with soil (31, D. Sugar, personal observation). Recovery of P. malorum from fruit surfaces or from orchard air during harvest in 1987 may be attributable to spores borne in dust raised during the harvest. Dry, dusty conditions were observed during the 1987 harvest. The lack of fruit contamination prior to harvest, together with the result that side rot developed in commercially packed but not in surface-sterilized fruit, indicates that latent infection of fruit while on the tree

Table III-6. Dynamics of transfer of spores of Phialophora malorum between surfaces of Bosc pears and a circulating water bath as a simulated packinghouse immersion tank.

| Treatment | Spores recovered /g peel | LSD (0.05) |
|---|---|---|
| 1. Fruit surface sprayed with P. malorum spore suspension ($10^4$/ml): | | |
| Check (no immersion) | 1806.1 +/- 457.6 | |
| After 2 min immersion | 739.1 +/- 517.1 | 712.1 |

| Treatment | Spores recovered /ml +/- sd |
|---|---|
| 2. Spores in water bath before and after immersion of fruit sprayed with P. malorum spore suspension ($10^4$/ml): | |
| Before immersion | 0 |
| After immersion | 40.8 +/- 31.8 |

| Treatment | Spores recovered /g peel +/- sd |
|---|---|
| 3. Spores on surface of sterilized fruit before and after immersion in water bath containing P. malorum ($10^4$/ml): | |
| Before immersion | 0 |
| After immersion | 764.9 +/- 205.7 |

is unlikely. It is possible that the intended meaning of McColloch (30), in claiming that apples become infected by P. malorum while on the tree, was that they become infested on the tree, which is not unreasonable since we recovered P. malorum from harvested pears in bins while in the orchard. The disease may also behave differently in apple, or in different climates or soil conditions. The fruit bagging experiment provided evidence that infestation while on the tree does not occur, since no side rot developed in either treatments or controls in either year of study. Fruit in this experiment may have escaped infestation by soilborne P. malorum, however, or other conditions for infection, such as injury during harvest or handling (Chapter 4), may have been lacking.

Propagule levels of P. malorum in orchard soil have been shown to be relatively high during the harvest period (Chapter 1). Thus the likelihood of soil on harvest bins or in dust bearing propagules of P. malorum is highest at this time.

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 such 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 (42), but as shown in this and other studies (10,11,42,46), significant numbers of viable spores may also be present. Fruit receive a fresh water rinse to remove flotation salt and SOPP upon removal from the immersion tank, which may also remove spores. 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, while other fruit become infested in the immersion tank with spores transferred from orchard-infested fruit or brought into the tank in soil contaminating harvest bins.

# Chapter IV.

The importance of wounds as infection courts for postharvest decay of pear by Phialophora malorum and the role of hydrostatic pressure in spore penetration of wounds.

## INTRODUCTION

Side rot, caused by Phialophora malorum (Kidd and Beaum.) McColloch is an important postharvest disease of pear in southern Oregon (6,7,43). It is a disease of pears in long-term storage, rarely observed before 3 mo at -1 C, and more commonly appearing 4-6 mo after pears are placed in cold storage (43). Side rot is not controlled by any fungicide currently registered for postharvest use on pear (7,45). It has been shown to be resident in orchard soil (Chapter 1), and may be transported into packinghouses in dust contaminating fruit or soil adhering to harvest bins (Chapter 3). 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 following immersion in tanks of water containing a flotation salt and sodium o-phenyl phenate as a disinfestant (16,26,40,42). Immersion tanks often harbor spores of decay fungi (8,10,11,39,41, Chapter 3), and may be important sites of

redistribution of inoculum of P. malorum in pear (Chapter 3).

Bertrand et al (7) observed that P. malorum can penetrate apparently unbroken pear skin, and demonstrated that this occurred at 20 and 10 but not at 5 C. Pears are stored commercially at -1.1 to 0 C (16,33,35). Several studies of P. malorum as causal agent of postharvest decay of apple have noted that lesions tend to be centered around lenticels or small wounds (19,20,25,29), and Penicillium expansum Link ex Thom has been reported to infect via lenticels as well as wounds (1,2,4). However, McColloch (30) noted "it is difficult to determine in many cases whether the point of entrance is through a lenticel or through cuticular cracks."

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

## MATERIALS AND METHODS

**Lenticels as infection courts.** The ability of P. malorum to infect pears via lenticels was tested by infectivity titration in cold storage. Plastic tubes approximately 6 mm long by 4 mm internal diameter were

coated on the bottom edge with vaseline and centered about lenticels on the surfaces of Bosc pears stabilized on cupped fibreboard 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 spore concentration was determined by counting on a hemacytometer. The tubes were then filled with 0.1 ml of distilled water containing either 0, 10, $10^2$, $10^3$, $10^4$, $10^5$, $10^6$ conidia of P. malorum. Tubes were placed over lenticels on 25 pears for each spore concentration. As a check on the infectivity of the inoculum, five additional fruit 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 by 2 mm deep. The trays were covered with polyethylene bags and held at 0 C. The fruit were examined periodically for development of decay over a 6-mo period. The experiment was repeated the following year.

In order to evaluate the accessibility of Bosc pear lenticels to infection, 25 fruit were soaked for 24 hr in water containing 250 mg/L methylene blue (13) and rinsed in fresh water. Thin sections were cut through lenticels into fruit cortical tissue and then examined microscopically to determine the extent of dye penetration via the lenticel. A total of 185 lenticels were examined in this manner.

The effect of bruising on permeability of lenticels and interlenticellular epidermal areas was examined by dropping a 142 g round-headed steel bolt through 1.9-cm diameter PVC tubes of 5, 10, 15, or 20 cm length centered over marked lenticels or interlenticellular epidermal areas on each of 10 Bosc pears per treatment. Fruit were soaked for 24 hr in a methylene blue solution as above, then examined microscopically for dye penetration.

**Effect of hydrostatic pressure on lenticel or wound penetration.** When bins of fruit are immersed in packinghouse dump tanks, fruit at different locations in the bins are exposed to hydrostatic pressure corresponding to the height of water above them, ranging from a few cm to approximately 1 m. 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 PVC tube 110 cm high by 10 cm diameter was filled with water to 100 cm. A 125-cm wooden dowel was fashioned into a fruit plunger by attaching a perforated 8-cm diameter plastic disk to one end, with a wire-mesh basket large enough to carry 1 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, increased agitation of solutions in the tube when the plunger 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/ml as determined by counting on a hemacytometer. Bosc pears were surface sterilized 5 min in 0.5 % sodium hypochlorite (NaOCl) and puncture-wounded to a depth of 2 mm with steel needles or finishing nails to give wound diameters of 0.4, 0.5, 1, 2, or 6 mm. Fifteen unwounded fruit and fruit with each size wound were individually immersed for 15 sec, 2 min, or 5 min in the spore suspension at depths from 0 - 100 cm in 10 cm increments. Each fruit was then rinsed for 15 sec 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 microscopic examination of the shape and size of conidiogenous cells and conidia (30).

The above experiment was repeated with the tube water containing conidia of Penicillium expansum, causal agent of blue mold decay of apple and pear (1,3,14,34), immersed to depths of 0, 20, 40, 60, 80, and 100 cm. Additionally, lenticels of Bosc pears were bruised by dropping a weight from 0, 5, 10, 15, or 20 cm as described above, and immersed in the P. malorum spore suspension to depths of 0, 10, 20,

40, 60, 80, or 100 cm. All fruit were stored for 4 mo at 0 C and evaluated as above.

**Cullage survey.** In October, 1988, approximately 100 Bosc pears graded as cull were collected from conveyor belts in each of 3 packinghouses in Medford, Oregon. Samples were taken to the laboratory and examined under a disssecting microscope for the cause of cullage. The size of puncture wounds, when present, was determined by averaging diameters measured along the longest and shortest diameter of the wound.

**Effect of wound exudates on spore germination.** An experiment was conducted to evaluate whether or not wounds in pear fruit, in addition to being an infection court for P. malorum, provide stimulus for spore germination. Five Bosc pears, surface-sterilized in NaOCl, were wounded with a 6-mm diameter nail to a depth of 3 mm, then the fruit were individually soaked for 18 hr in 250 ml distilled water. After removing the fruit from the water, 1 ml of a spore suspension of P. malorum ($10^4$/ml) was added to the water, and the solutions were incubated at 15 C. After 2, 4, 6, and 8 days incubation, five 0.1 ml aliquots were removed from the solutions and examined microscopically to determine the percent of spores which had germinated. Spores were considered germinated if germ tubes were greater than one-half the length of the spore (47). The same procedure also was followed with unwounded fruit and fruit from which

extracuticular wax had been removed by a 20 sec dip in chloroform (27), followed by a fresh water rinse. Spores of P. malorum were also added to distilled water in which fruit had not been soaked. The soluble solids ($^{o}$ Brix) of each soaking solution was determined using a hand-held refractometer (American Optical Co., Keene, NH Model 10421).

**Relationship of inoculum concentration to disease severity at wounds.** Fruit of four important commercial cultivars of pear, Bosc, Comice, Anjou, and Bartlett were harvested at optimum maturity, surface-sterilized in 0.5 % NaOCl, and wounded with a 6-mm diameter nail to a depth of 3 mm. Wounds were then filled with 0.05 ml of a suspension of spores of P. malorum in distilled water, to give either 0, 10, $10^2$, $10^3$, $10^4$, or $10^5$ spores per wound. Twenty fruit of each cultivar with 5 wounds/fruit were treated with each spore concentration, then stored for 3 mo at 0 C. After storage, lesion diameters at wounds were measured and lesion areas calculated.

**Effect of growth medium on virulence of P. malorum.** Colonies of P. malorum were established on PDA (Difco) and on water agar, each prepared with and without 200 ml/L Bosc pear juice added before autoclaving. After 3 weeks, spores were washed from the colonies and wounds in Bosc pears filled with either 0, 10, $10^2$, $10^3$, or $10^5$ spores/wound from each growth medium. After 3 mo at 0 C, lesion diameters were measured and lesion areas calculated.

**Statistical analyses.** Data from experiments on the effects of hydrostatic pressure on disease incidence and on the relation of inoculum concentration to disease severity at wounds were analyzed by linear regression analysis using Statgraphics software (Statistical Graphics Corporation, Rockville MD). In experiments on spore germination and on the effect of growth medium on virulence of P. malorum, treatment means were compared by Fisher's protected least significant difference (LSD) test, using Number Cruncher Statistical System software (J.L. Hintze, Kaysville UT).

## 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 IV-1). Of the 185 lenticels dissected following dye treatment, only 4 were observed to be "open" as indicated by dye penetration of parenchymatous tissue beneath the lenticel. Wounding of lenticels increased penetration by dye solution, and penetration was induced at lenticels at lower impact than in interlenticellular epidermal areas (Table IV-2). Wounding did not affect penetration of lenticels by

Table IV-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 | |
|---|---|---|
| | Unwounded (25 fruit) | Punctured (5 fruit) |
| 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 |

1 Plastic cylinders were centered over lenticels, sealed to fruit surfaces with petroleum jelly, and filled with 0.1 ml *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 incubation at 0 C.

Table IV-2. Penetration of lenticels or interlenticellular areas in Bosc pears by a methylene blue solution after bruising.

| Drop height (cm)[1] | Number penetrated / 10 fruit | |
|---|---|---|
| | Lenticels | Interlenticellular areas |
| 0 | 0 | 0 |
| 5 | 0 | 0 |
| 10 | 0 | 0 |
| 15 | 2 | 0 |
| 20 | 7 | 2 |

[1] A 142 g round-headed steel bolt was dropped through tubes of various lengths onto marked fruit lenticels or interlenticellular areas prior to soaking fruit 24 hr in 250 mg/L methylene blue solution.

spores of P. malorum at any level of hydrostatic pressure tested (Table IV-3).

**Effect of hydrostatic pressure on lenticel or wound penetration.** A significant interaction between immersion depth and wound size in infection of Bosc pears by P. malorum and was observed (Fig. IV-1). Infection did not take place at any depth in unwounded fruit. At wound diameters of 0.4 and 0.5 mm, infection occurred at 0-20% of the wounds, and infection increased with greater immersion depth. At wound diameters $\geq$ 1 mm infection took place at all immersion depths, and infection occurred in 68 to 100% of the wounds. Infection of wounds of 1-mm diameter was strongly influenced by immersion depth, while the effect of immersion depth was not significant in wounds of 2-mm or 6-mm diameter according to linear regression analysis (Fig. IV-1). A similar relationship between wound size and immersion depth was observed using spores of Penicillium expansum as inoculum (Fig. IV-2).

**Cullage survey.** Of various reasons for cullage determined in examination of fruit from three packinghouses, punture wounding was the most frequent (Table IV-4). Puncture wounding accounted for 81.5, 69.1, and 50.0% of the cullage in the three packinghouses, respectively.

**Effect of wound exudates on spore germination.** Spore germination was more rapid and a significantly greater percentage of spores germinated in water in which wounded

Table IV-3. Interaction of degree of bruising and depth of immersion in a spore suspension on lenticel infection of Bosc pears by *Phialophora malorum*.

| Immersion depth (cm)[b] | Number of infections/15 fruit | | | | |
|---|---|---|---|---|---|
| | Drop height (cm)[a] | | | | |
| | 0 | 5 | 10 | 15 | 20 |
| 10 | 0 | 0 | 1[c] | 0 | 0 |
| 20 | 0 | 0 | 0 | 1[c] | 0 |
| 40 | 0 | 0 | 0 | 0 | 0 |
| 60 | 0 | 0 | 0 | 0 | 0 |
| 80 | 0 | 0 | 0 | 0 | 0 |
| 100 | 0 | 0 | 0 | 0 | 0 |

[a] Lenticels bruised by a round-headed 142 g steel bolt impacting lenticels from various heights.

[b] After bruising, fruit were immersed 2 min at various depths in water containing $10^5$ spores of *P. malorum* per ml.

[c] Infections centered about skin breaks rather than lenticels.

Fig. IV-1. Effects of immersion depth and wound diameter on infection of pear fruit by Phialophora malorum following immersion in water containing $10^4$ conidia/ml . Fruit were held at indicated depths for 2 min, rinsed in fresh water, and stored 3 months at $0^{\circ}C$ prior to evaluation of lesion development. Values represent means of 15 fruit per treatment. Regression analysis was performed separately within each wound diameter category. No significant relationships existed between immersion depth and infection at wound diameters $\geq$ 2 mm ($P = 0.05$). Regression of percent wound infection on immersion depth with wound diameters of 0.4, 0.5, and 1 mm were described, respectively, by the following equations: $y = -2.21 + 0.101\ x$, $R^2 = .48$, $p < 0.05$; $y = -4.43 + 0.214\ x$, $R^2 = .78$, $p < 0.01$; $y = -2.22 + 0.937\ x$, $R^2 = .96$, $p < 0.001$.

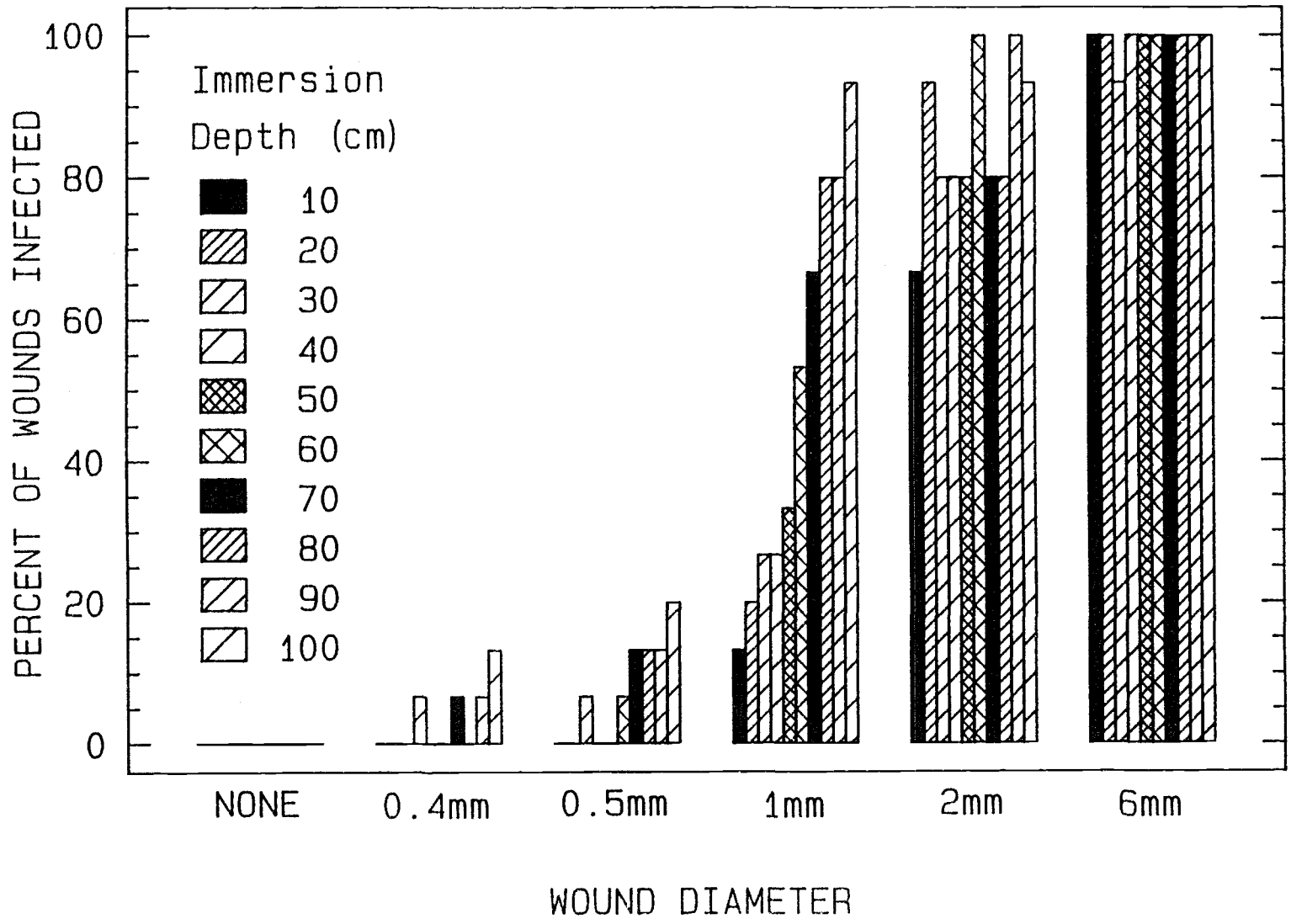
100
80
60
40
20
0
PERCENT OF WOUNDS INFECTED
Immersion
Depth (cm)
10
20
30
40
50
60
70
80
90
100
NONE
0.4mm
0.5mm
1mm
2mm
6mm
WOUND DIAMETER

Fig. IV-1.

Fig. IV-2. Effects of immersion depth and wound diameter on infection of pear fruit by Penicillium expansum following immersion in water containing $10^4$ conidia/ml . Fruit were held at indicated depths for 2 min, rinsed in fresh water, and stored 3 months at $0^{o}C$ prior to evaluation of lesion development. Values represent means of 15 fruit per treatment. Regression analysis was performed separately within each wound diameter category. No significant linear relationships existed between immersion depth and infection at wound diameters 0.5, 2, or 6 mm ($P = 0.05$). Regression of percent wound infection on immersion depth with wound diameters of 0.4, and 1 mm were described, respectively, by the following equations: $y = 32.38 + 2.48\ x$, $R^2 = .76$, $p < 0.05$; $y = 7.86 + 0.916\ x$, $R^2 = .90$, $p < 0.01$.

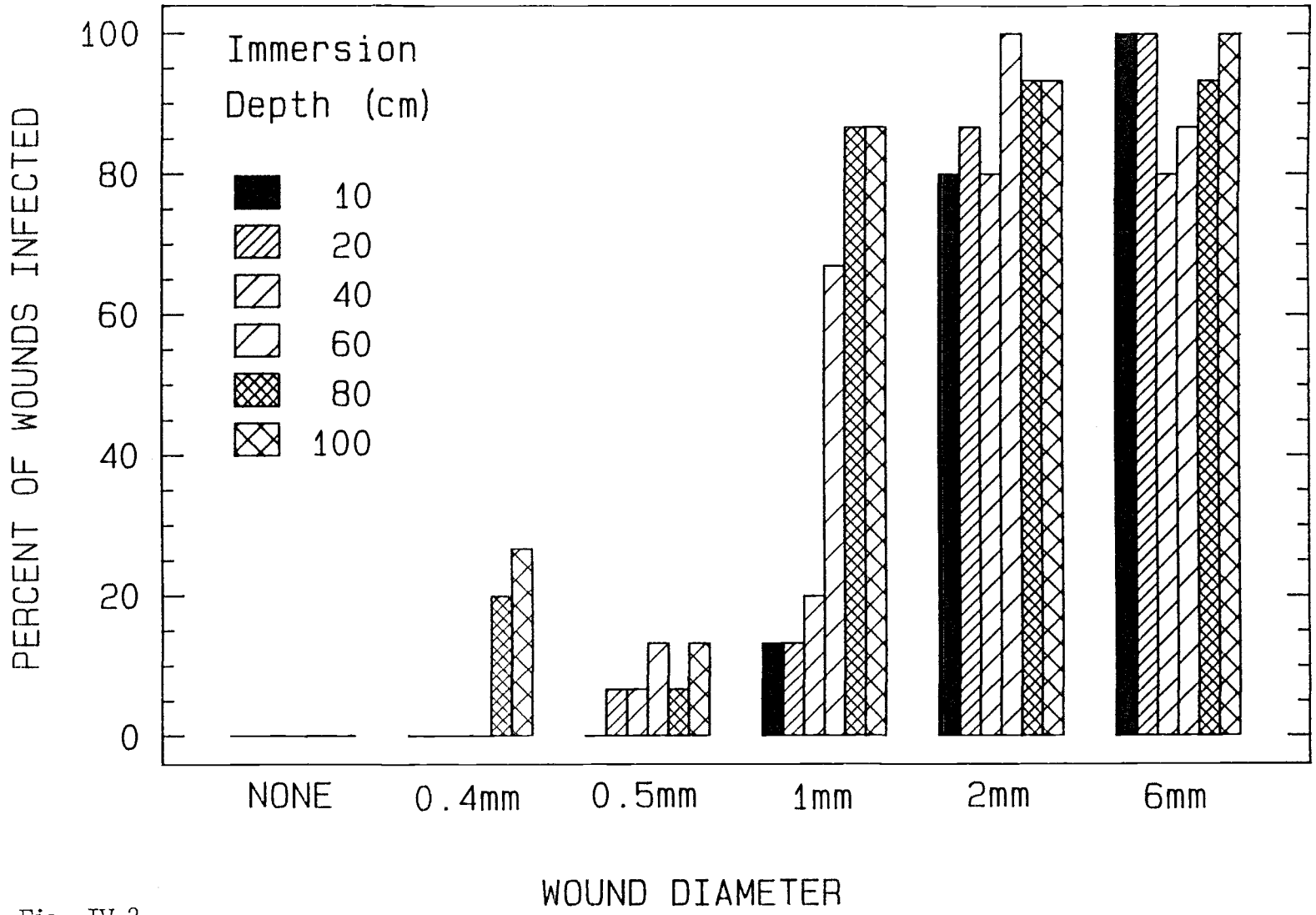
PERCENT OF WOUNDS INFECTED
100
80
60
40
20
0
Immersion
Depth (cm)
10
20
40
60
80
100
NONE
0.4mm
0.5mm
1mm
2mm
6mm
WOUND DIAMETER

Fig. IV-2.

Table IV-4. Survey of cullage in Bosc pears from three packinghouses in Medford, Oregon.

| Cause of cullage | % of cull fruit[a] Packinghouse 1 | 2 | 3 |
|---|---|---|---|
| Puncture >5 mm | 18.5 | 10.9 | 14.3 |
| 3-5 | 27.8 | 23.6 | 10.7 |
| 1-3 | 20.4 | 25.5 | 12.5 |
| 0.5-1 | 14.8 | 9.1 | 12.5 |
| Puncture total | 81.5 | 69.1 | 50.0 |
| Gash | 5.5 | 5.5 | 5.4 |
| Hail | 3.6 | 7.3 | 12.5 |
| Stem break | 5.6 | 3.6 | 8.8 |
| Sunburn | 1.9 | 0.0 | 5.4 |
| Limbrub | 1.9 | 9.1 | 12.5 |
| Other | 0.0 | 5.4 | 5.4 |
| Total | 100.0 | 100.0 | 100.0 |

[a] 100 fruit graded as cull were collected from each packinghouse and examined under a dissecting microscope for cause of cullage.

fruit had been soaked than in water alone or water in which unwounded pears had been soaked (Fig. IV-3). After 2 days, 32.0% of the spores had germinated in water in which wounded fruit had been soaked, while 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 $0^{o}$, indicating that the concentration of dissolved sugars was not detectably increased by the treatments.

**Relationship of inoculum concentration to disease severity at wounds.** The relationship of inoculum concentration to disease severity at wounds was cultivar-dependent (Fig. IV-4). Relatively small lesions developed at inoculum concentrations of 10 or $10^2$ spores/wound in all cultivars. The rate of lesion expansion increased sharply with increasing inoculum concentration in Bosc and Comice fruit, and the relationship between inoculum dose and lesion size was similar in these cultivars. Lesions were smaller at corresponding inoculum levels in Bartlett than in Bosc or Comice fruit, and were smallest in Anjou.

**Effect of growth medium on virulence of P. malorum.** Inoculation of Bosc pear wounds with spores from colonies grown on water agar and water agar + pear juice resulted in

Fig. IV-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 by 6 mm diameter, or had epicuticular wax removed by a 20 sec dip in chloroform. Fruit were then soaked 18 hr in 250 ml distilled water. After fruit 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 ($P=0.05$).

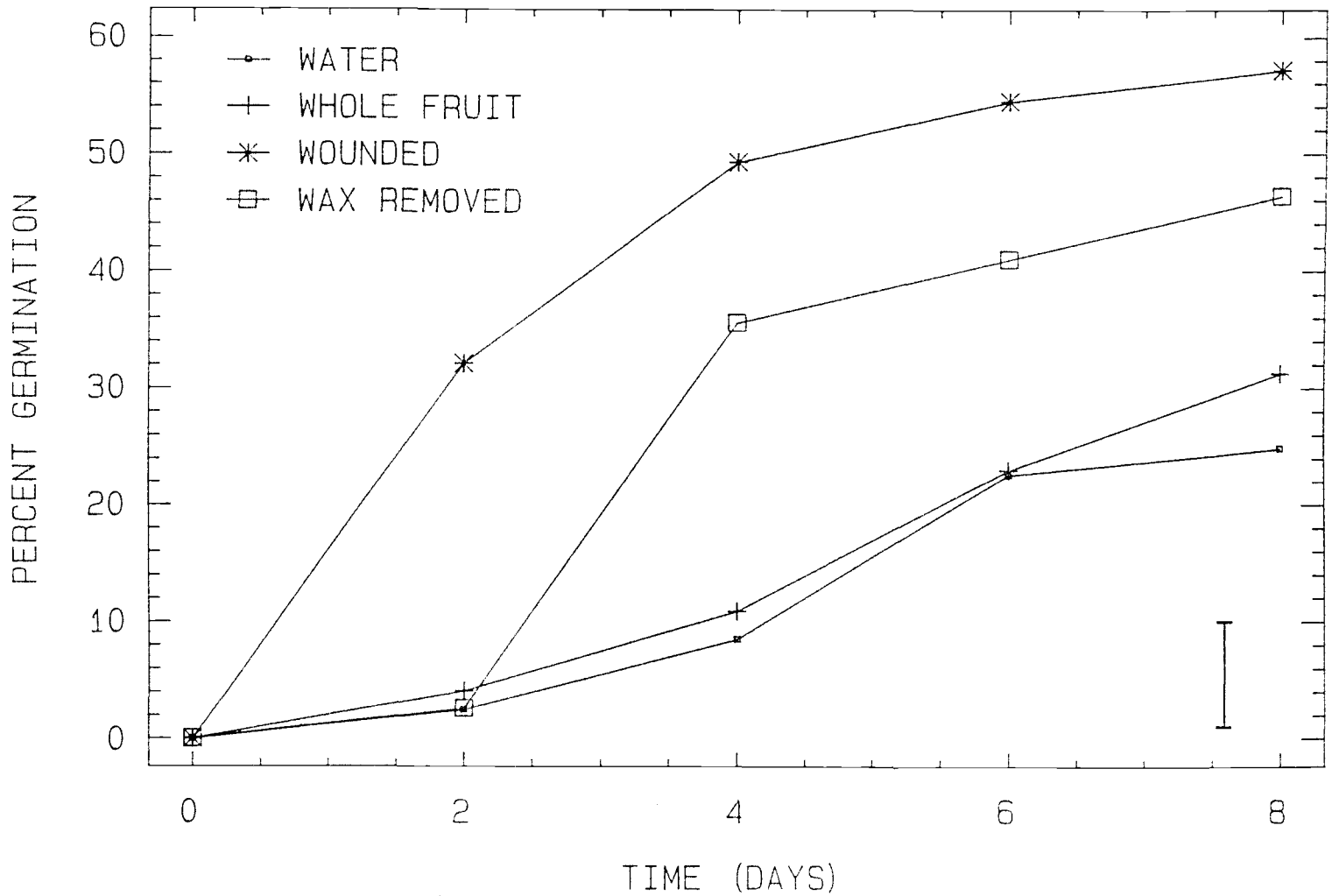
WATER
WHOLE FRUIT
WOUNDED
WAX REMOVED
PERCENT GERMINATION
60
50
40
30
20
10
0
0
2
4
6
8
TIME (DAYS)

Fig. IV-3.

Fig. IV-4. Relationships between inoculum dose and disease severity in fruit of major pear cultivars wound-inoculated with spores of Phialophora malorum. Surface-sterilized fruit were wounded 3 mm deep by 6 mm diameter and each wound inoculated with the indicated number of conidia in 0.05 ml sterile water. After 3 mo storage at 0 C, lesion diameters were measured and areas calculated. Values represent means of five replicate fruit lots of 10 fruit with one wound per fruit. Bosc: $y = 4.3647 + 0.0103\ x - 9.3575E\text{-}8\ x^2$, $R^2 = 0.982$. Comice: $y = 9.010 + 0.0087\ x - 7.6682E\text{-}8\ x^2$, $R^2 = 0.924$. Bartlett: $y = 1.7045 + 0.0006\ x$, $R^2 = 0.996$. Anjou: $y = 0.0710 + 0.0001\ x$, $R^2 = 0.994$.

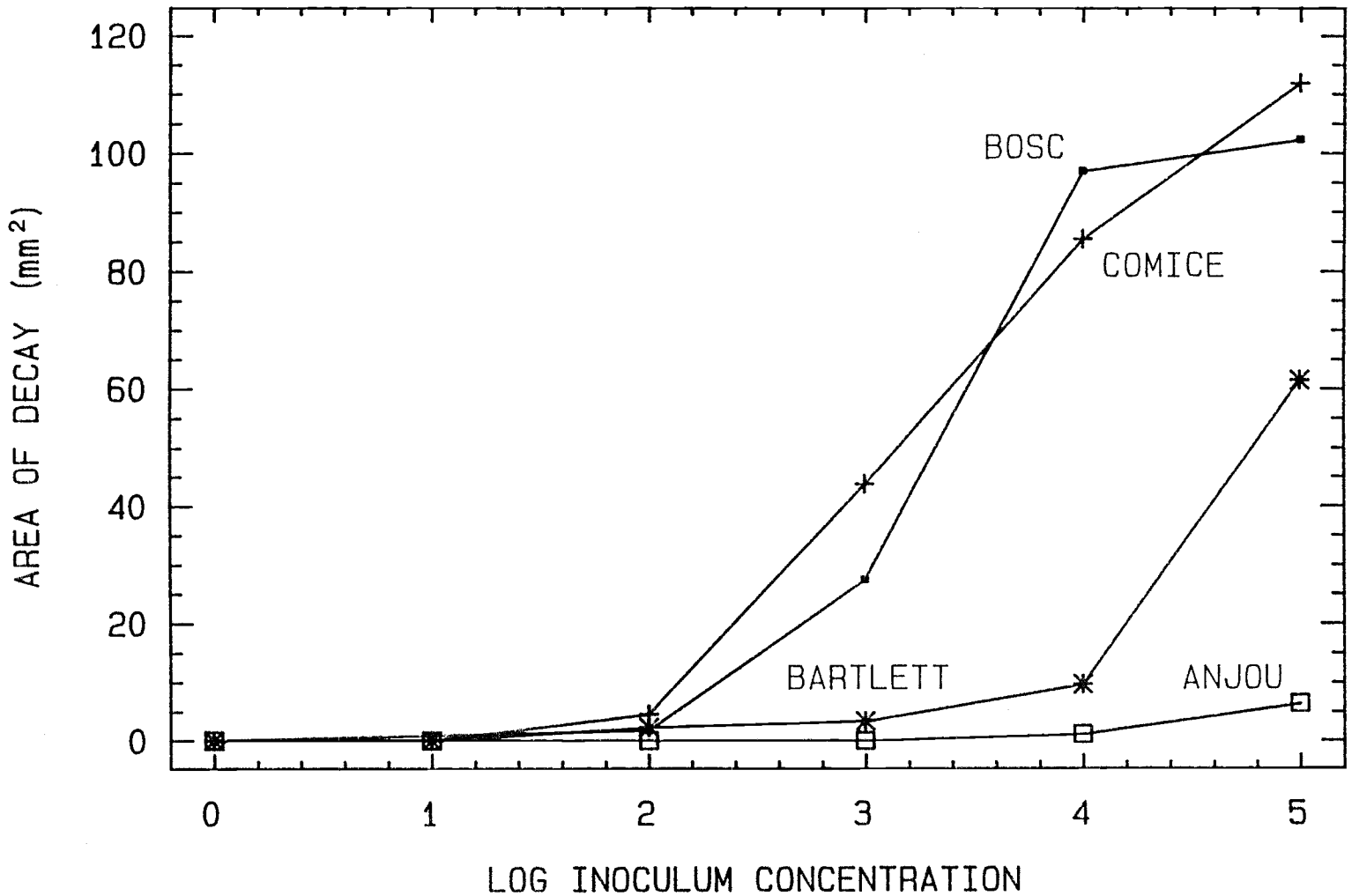
120
100
80
60
40
20
0
AREA OF DECAY (mm²)
BOSC
COMICE
BARTLETT
ANJOU
0
1
2
3
4
5
LOG INOCULUM CONCENTRATION

Fig. IV-4.

significantly smaller lesions than spores from colonies grown on PDA or PDA + pear juice at an inoculum concentration of $10^2$/wound (Table IV-5). However, this effect was not seen at the other inoculum concentrations. There was a consistent trend towards greater lesion areas in treatments containing pear juice as compared to similar treatments without pear juice, but the differences were not significantly different.

## DISCUSSION

It is difficult to say unequivocally whether or not 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 herein, 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. As 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

Table IV-5. Effect of growth medium on virulence of *Phialophora malorum*.

| Growth medium[a] | Lesion area ($mm^2$) Log inoculum concentration 1 | 2 | 3 | 5 |
|---|---|---|---|---|
| PDA (Difco) | 0.64 | 20.76 | 31.64 | 53.76 |
| PDA + pear juice[b] | 1.41 | 24.48 | 33.55 | 59.27 |
| Water agar | 0.10 | 11.64 | 26.72 | 52.18 |
| Water agar + pear juice[b] | 0.89 | 12.45 | 30.25 | 53.75 |
| LSD (0.05) | 4.33 | 8.30 | 9.28 | 13.39 |

a Spores were washed from 3 wk old colonies on each medium and inoculated into Bosc pear wounds at a range of inoculum concentrations. Lesions were measured after 3 mo storage at 0 C.

b 200 ml Bosc pear juice per liter of medium.

species, cultivar, and climatic or cultural conditions (13). Since bruising more effectively induced dye penetration at lenticels than at interlenticellular areas, lenticels could be more vulnerable to infection under certain (unknown) conditions than other epidermal areas. Clements (13) examined methylene blue penetration of lenticels in 13 apple cultivars, and found that the proportion of open lenticels varied with cultivar from 4.8-29.8%. English et al (19) dissected blue mold (P. expansum) lesions in Delicious apple apparently initiated via lenticels and concluded that 63% of the lesions examined were true lenticel infections. However, while also noting lenticel infection of Delicious apple 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 took place 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 so infected was 33 and 40%, respectively, further suggesting differential condition of lenticels. A factor which may have confounded their study, however, is that Bosc pears ripen and cell senescence may be advanced within 3 wk at incubation temperatures of 20 or 10 C (23,33,35), so lenticel susceptibility after 3 wk at these temperatures may

not be indicative of their 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 which may increase incidence of fine punctures in the fruit during harvest or in wind-driven movement of fruit on the tree (44). While 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 of 0.4- and 0.5-mm diameter were infected after immersion to 100 cm. The percent of infection was increased with greater hydrostatic pressure due to depth in the immersion tank (Fig. IV-1). Results of the cullage survey demonstrate that wounds of various sizes occur during commercial handling of pears. It has been noted that 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 (44).

Exudation via wounds stimulates spore germination in P. malorum (Fig. IV-3), and may affect occurrence of infection at wound sites. While 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 may also be due to leakage of biochemicals which require methods other than refractometry to detect. Results of infectivity

titration (Fig. IV-4) at wounds indicate that Bosc and Comice pears are the most inherently susceptible to advance of P. malorum through fruit tissue of the four major pear cultivars grown in the Pacific Northwest. Reports of the relationship between inoculum dose and incidence of several postharvest diseases of pear and apple have described quadratic relationships (10,39). The relationships between inoculum dose and disease severity were found in this study to be quadratic in the more susceptible cultivars (Bosc and Comice) and linear in the less susceptible cultivars (Bartlett and Anjou) over the range of inoculum concentrations tested (Fig IV-4).

The relationship between wound size and immersion depth (Fig. IV-1) suggests that their interaction is based on the amount of force required for the spore suspension to penetrate the wound cavity. Infection of wounds $\geq$ 2 mm diameter by P. malorum was independent of immersion depth, whereas infection of wounds $\leq$ 1 mm diameter was dependent on immersion depth. Increased penetration of apple cores by dip solutions, and consequent greater risk of core rot, was correlated with increasing immersion depths (R. Holmes, seminar for apple and pear growers, July 1987, Dept. of Agriculture and Rural Affairs, Victoria, Australia). 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 atmosphere or 9.806 kPa (12). While the precise relationship between wound size and immersion depth in a fruit immersion tank may be influenced by the presence of dissolved and suspended materials which affect the specific gravity and surface tension of the solution (8), the results of this study indicate that depth of immersion may be a significant factor in the incidence of fruit infection by postharvest decay fungi.

# CONCLUSIONS

*Phialophora malorum* is a soil-dwelling fungus whose role as a postharvest pathogen of pear is dependent upon passive dispersal with infested soil. While a limited amount of fruit may become infested prior to entering the packinghouse, the practice of handling fruit in immersion dump tanks facilitates both infestation of previously uninfested fruit by inoculum redistribution, and penetration of inoculum into fruit wounds, which are the principal sites of infection. A generalized diagram of the side rot disease cycle is shown in Fig. V-1.

*P. malorum* survives in soil under a wide range of conditions. However, survival is greatest in cool, moderately dry soil, and in soil of near-neutral pH. Under warm, moist conditions generally favorable to microbial activity, *P. malorum* survives in soil at relatively low population levels, suggesting it is a weak competitor. *P. malorum* is not a primary colonizer of fallen fruit on the orchard floor, but populations increase in response to nutrient release subsequent to fruit decay by other microorganisms.

*P. malorum* can also survive saprophytically on the bark of pear trees. This survival does not appear important in the disease cycle, however, since fruit did not become

Fig. V-1. Diagramatic representation of the disease cycle of side rot of pear.

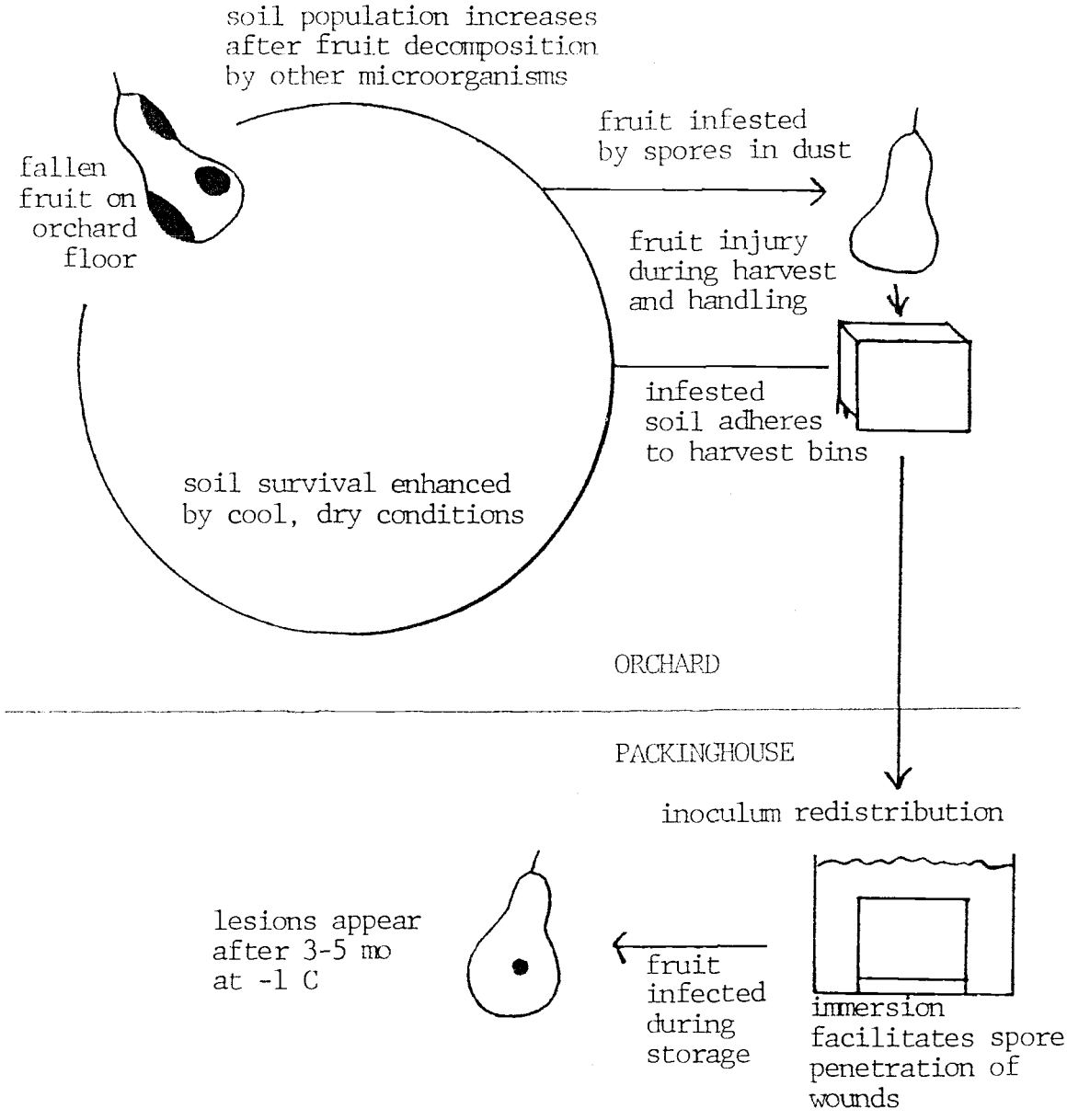
soil population increases
after fruit decomposition
by other microorganisms
fallen
fruit on
orchard
floor
fruit infested
by spores in dust
fruit injury
during harvest
and handling
infested
soil adheres
to harvest bins
soil survival enhanced
by cool, dry conditions
ORCHARD
PACKINGHOUSE
inoculum redistribution
lesions appear
after 3-5 mo
at -1 C
fruit
infected
during
storage
immersion
facilitates spore
penetration of
wounds

Fig. V-1.

infested with P. malorum prior to harvest, and uninoculated bark areas did not become infested by spores from inoculated areas on the same trees.

The ability of P. malorum to cause cankers in pear trees is dependent upon injury to the bark. Cankers are non-perrenating, and P. malorum is not recoverable from canker tissue for more than one growing season. The development of tree cankers does not appear to be important in the side rot disease cycle. Cankered areas resulting from inoculations of wounded bark heal after the fungus has died out.

Wounds are the primary courts for infection of pear fruit by P. malorum. Lenticel infection could not be induced in Bosc pears despite inoculation under treatment conditions including bruising, hydrostatic pressure, and long-term exposure over a broad range of spore concentrations. The size of wounds and the depth of immersion in dump tanks are significant factors affecting wound penetration by packinghouse tank solutions carrying spores of decay pathogens. Wound exudates stimulate germination of spores of P. malorum.

Several available or potential orchard and packinghouse actions to reduce side rot incidence are suggested by these conclusions. Foremost are practices to reduce inoculum levels, such as removal of fallen fruit from the orchard floor, avoiding dusty conditions during harvest, avoiding soil contamination of bins during harvest or cleansing of

bins prior to packinghouse entry, minimizing injuries to fruit during harvest, and improving disinfestant activity in immersion tank solutions. In addition, practices or treatments which stimulate wound healing, and reducing depth of immersion during dumping are likely to significantly aid disease control. The relative weakness of P. malorum as a competitor in colonization of pear tissue suggests that biological control would be effective.

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