

Population Dynamics of *Mucor piriformis* in Pear Orchard Soils as Related to Decaying Pear Fruit

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ABSTRACT

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The population dynamics of *Mucor piriformis* in soil in four pear orchards were studied over a 3-yr period. Populations of sporangiospores fluctuated in an annual cyclic pattern, with a sharp increase occurring about 1-3 mo after harvest. Population densities increased from less than 10^2 to between 10^3 and 2×10^3 sporangiospores per cubic centimeter of dry soil, then rapidly declined between December and February, and usually remained below 10^2 sporangiospores per cubic centimeter throughout the summer. In soil kept free of vegetation and fruit, the population density of sporangiospores declined rapidly from 1.5×10^5 per cubic centimeter and remained below 10^3 per cubic centimeter for

more than 2 yr. Addition of pear fruits to this soil resulted in an increase from 14 to 9.6×10^3 spores per cubic centimeter between September 1986 and January 1987. In controlled plots, soils amended with pear fruit showed a significant increase in propagule population densities. However, in commercial orchards, the relationship between density of fruit on the orchard floor and population densities of sporangiospores in soil was less clear. The importance of additional factors affecting population densities of *M. piriformis* in pear orchards, including insect and rodent vectors, spread of infected fruits by mowing and birds, and favorable soil temperature, are discussed.

Mucor piriformis Fischer is a soilborne fungus that enters packinghouses in soil adhering to fruit bins (7). Infection of fruit is believed to occur in the dump tank, with rot developing in storage (1). Postharvest losses caused by *M. piriformis* can be a serious problem in pears (*Pyrus communis* L.) (1,4) and peaches (*Prunus persica* L.) (11). Two disinfectants, chlorine and sodium *o*-phenylphenate, are used in dump tanks to reduce the number of spores and reduce decay (1). Application of benomyl before storage of fruit gives adequate control of *Botrytis cinerea* and *Penicillium expansum*; however, no registered fungicides are effective against *M. piriformis*, and other control measures are needed.

Michailides and Ogawa (5) showed that the sporangiospores of *M. piriformis* are the surviving propagules and studied their longevity under different soil temperatures and moistures. Although zygospores are present in the life cycle of *M. piriformis* (8), they rarely are found in nature (9), and their role in survival of the fungus is unknown. In a study in the Hood River Valley, natural population densities of *M. piriformis* were higher in certain pear orchard soils than in others (12). Population densities were higher 2 mo after harvest than at harvest in September because pear fruits left on the ground after harvest were colonized by *M. piriformis* (7). In laboratory experiments, crushed leaves of peach, ryegrass, and chickweed also supported sporulation, germination, and growth of *M. piriformis* (6).

Our research was initiated to study the long-term population dynamics of sporangiospores of *M. piriformis* in natural orchard soils, and to determine the effect of pear fruits on populations in soil in which these substrates were added or withheld. The density of fruit on the orchard floor and the colonization of this fruit by *M. piriformis* also were studied. A portion of this study has been published (2).

MATERIALS AND METHODS

Population dynamics in natural orchard soils. Four orchards in the Hood River Valley were used in this study. From July 1985 to June 1988, four soil samples were collected monthly from the top 5 cm of soil at the drip line at the north, east, west, and south side of each of three pear trees in each orchard. The 12 soil samples from each location were combined and mixed, and 100 g was removed for determination of soil moisture content by drying in an oven at 110 C for 24 hr. A 100-g sample was removed for sporangiospore determination. Soil was added to 200 ml of sterile distilled water, blended at slow speed (Osterizer 869, Oster Corp., Milwaukee, WI) for 1 min, and left to settle for 15 sec. A 0.1-ml subsample was spread on each of three plates containing potato-dextrose agar (Difco Laboratories, Detroit, MI) acidified with 1.5 ml of 85% lactic acid per liter (APDA). Plates were incubated at 5 C for 3-4 days, and the number of colonies of *M. piriformis* were counted by observing plates through transmitted light. Results were expressed as the number of sporangiospores per cubic centimeter of dry soil, based on bulk soil densities of 0.77, 1.11, 1.07, and 1.00 g/cm³ for soils 33 (loam), 95 (silt loam), 97 (loam), and 120 (silt loam), respectively. Numbers refer to orchards included in a previous study (12). Bulk densities were determined by the Oregon State University Soil Physics Laboratory.

Population dynamics in substrate-deficient orchard soil. Soil was obtained from the upper 5 cm of an established pear orchard at the Mid-Columbia Agricultural Research and Extension Center and screened through a 5-mm sieve to remove rocks and plant debris. Soil was mixed with a water suspension containing 1×10^6 sporangiospores of *M. piriformis* per milliliter to form a slurry containing approximately 1.2×10^5 sporangiospores per cubic centimeter of soil. Three kilograms of soil mix was placed in rectangular (25 × 17 × 6 cm) baskets made from galvanized hardware wire with 0.5-cm-square openings. Baskets were placed in the orchard before being filled with infested soil and were

positioned so the surface of the infested soil was level with the surface of the natural soil. Three replicates were prepared and placed outside of the orchard such that no fruit would fall into the plots. Plots were kept free of weeds and falling leaves throughout the study. Viable propagules were measured at the start of the experiment in September 1984 and monthly thereafter by removing a set of three soil cores of 2 cm diameter from each basket. Ten grams of soil from each basket was used to determine soil moisture as described above. Two grams of soil was diluted serially in distilled water and three subsamples of 0.1 ml plated on APDA plates as described earlier. Soil temperature at 5 cm depth was recorded when monthly soil samples were taken. On 24 September 1986, mature Anjou pear fruits were cut in half, and six pieces were placed with the cut surface down on half of each replicate plot. Soil from the plots amended and unamended with the pear halves was sampled separately until termination of the experiment in January 1987.

Soil amendment with pear fruits. In a preliminary experiment, the population density of *M. piriformis* in soil in a pear orchard remained low after addition of partially decomposed leaves and organic matter to the soil surface. However, an increase in population density during October through May was observed when pear fruits were placed on the soil surface. To study the effect of amendment of soil with pear fruits on population density of *M. piriformis* in more depth, we selected four orchards representing different areas within the Hood River fruit growing district. In each orchard, two treatments were established

6 September 1986 with four single-tree replications. In one of the treatments, all pears falling on the ground around the trees were removed, whereas in the other treatment, pears were added to an area of 1-m radius around the tree trunk to obtain about four pears per square meter. Soil population densities of *M. piriformis* were assayed every 6 wk until 18 May 1987 as described above. All results were expressed as the number of sporangiospores per cubic centimeter of dry soil.

Density and incidence of infection of pear fruits on the orchard floor. In each of four orchards, nine pear trees were selected and the area under the tree canopy was calculated based on the radius from the trunk to the terminal of major scaffold limbs. After commercial harvest in 1985, 1986, and 1987, pear fruits on the ground around each tree were counted. Fifty fruits in each orchard were examined visually every 14 days for decay caused by *M. piriformis*. The number of fruits per square meter and percent fruits infected were calculated, and the values were used to determine the number of infected fruits per square meter.

RESULTS

Population dynamics in natural orchard soils. The population density of sporangiospores in the orchard soils followed an annual, cyclic pattern (Fig. 1). In late autumn, about 1–3 mo after harvest, population densities increased sharply from below 4×10^2 to over 10^3 sporangiospores per cubic centimeter of orchard soil. Between December and February, the densities decreased

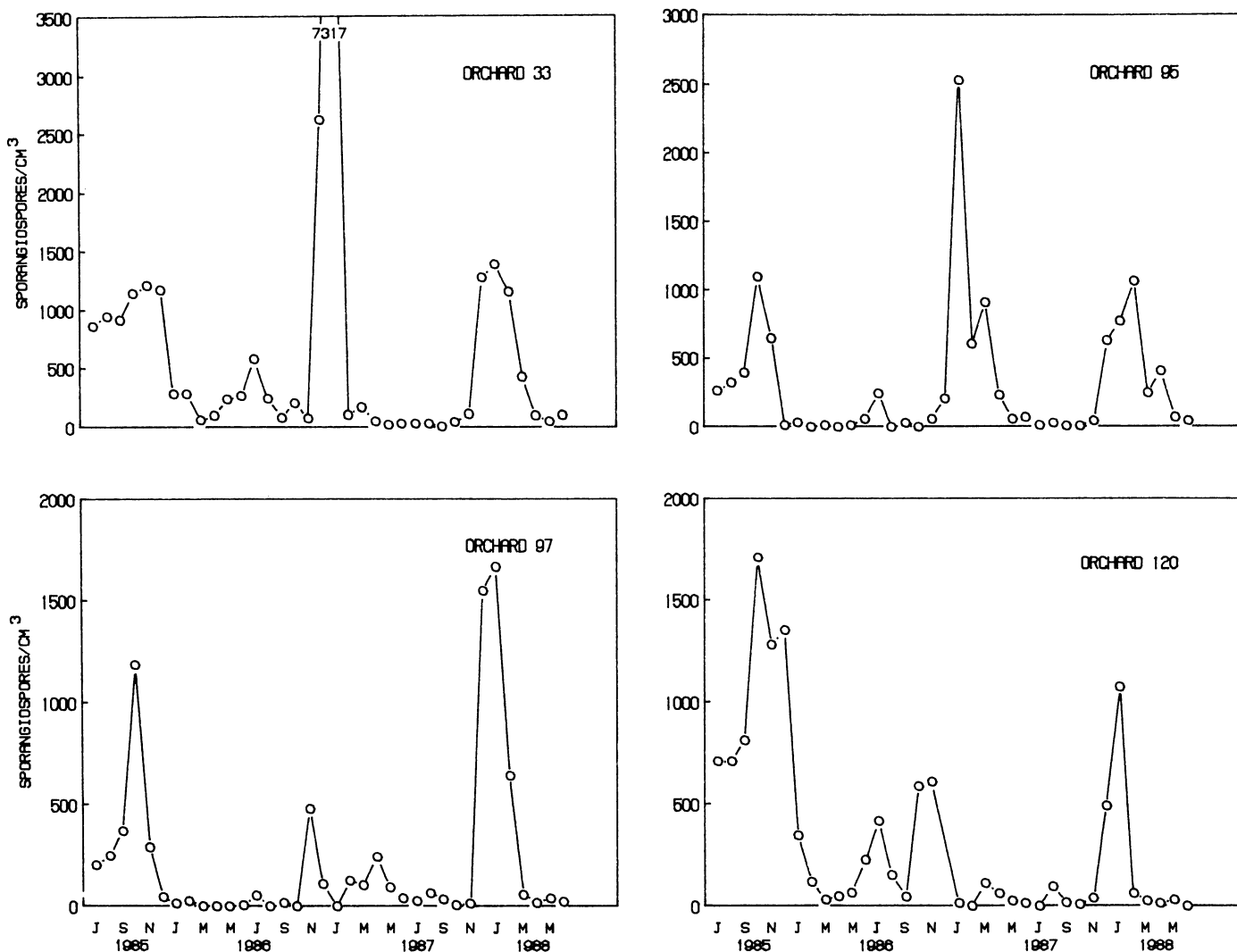


Fig. 1. Population densities of sporangiospores of *Mucor piriformis* in the upper 5 cm of soil in four orchards in the Hood River Valley of Oregon over a 36-mo period. Letters on the X-axis refer to months, beginning with July 1985. Each point is the average of three values.

dramatically to $< 4 \times 10^2$ spores per cubic centimeter of soil and remained low throughout the summer (Fig. 1). In 1986, population densities increased slightly in all orchard soils during the month of July but declined again in August.

Whereas soil in orchard 120 had the maximum population density during the first winter, orchards 33 and 97 soils had the highest population of sporangiospores during the second and third winters, respectively. In orchards 33 and 95, population densities in the soil were highest during the 1986–1987 winter, but orchards 97 and 120 were highest during the 1987–1988 and 1985–1986 winters, respectively (Fig. 1).

Population dynamics in substrate-deficient orchard soil. The average initial population density of *M. piriformis* in the soil

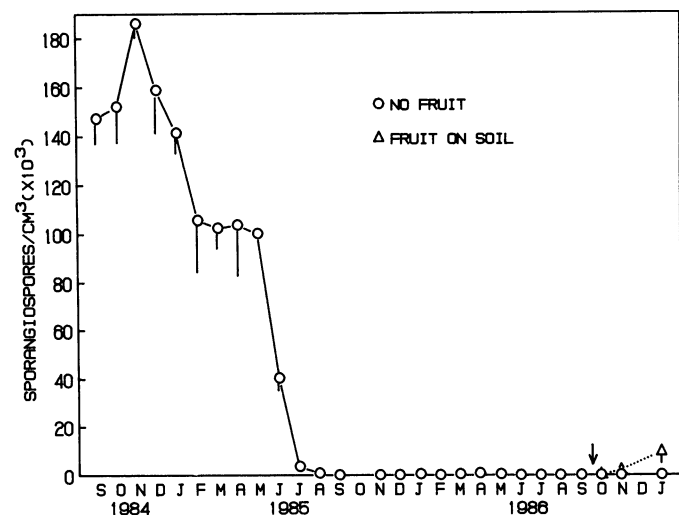


Fig. 2. Population densities of *Mucor piriformis* in infested soil outside of an orchard. Soil was kept free of fruit, weeds, and leaves. Fruit was added to half-plots on 24 September 1986 (arrow). Letters on X-axis refer to months, beginning with September 1984. Each point is the average of three values. Vertical bars represent standard error.

TABLE 1. Population densities of sporangiospores of *Mucor piriformis* in orchard soils with fruit (F) or no fruit (NF) from September 1986 to May 1987 in the Hood River Valley of Oregon

Date	Density of sporangiospores/cm ³ of soil ^{a,b}							
	33		95		97		123	
	F	NF	F	NF	F	NF	F	NF
Sept 6	533	54	13	4	2	0	19*	2
Oct 18	1,189	27	78	17	83**	0	3,840	56
Dec 1	2,639**	140	3,496*	98	5,348**	120	2,304	64
Jan 12	5,069**	248	820	180	4,095**	540	9,531**	32
Feb 23	2,368**	299	1,236	257	1,500	42	4,028**	42
Apr 6	1,084**	93	431	141	265	19	687**	52
May 18	617	51	6	8	133	3	180	12

^a33, 95, 97, and 123 designate soils from different orchards in the Hood River Valley of Oregon.

^bEach value represents the average of four replications. For each orchard soil type with fruit at each sampling date, numbers followed by * or ** are significantly different at $P = 0.05$ and $P = 0.01$, respectively, from population densities in soil with no fruit according to a nonpaired *t* test.

TABLE 2. Density and incidence of infection of Anjou pear fruits on the ground after harvest in four commercial orchards

Orchard	1985			1986			1987		
	Fruits/ ^a m ²	Fruits ^b infected (%)	Infected ^c fruits/ m ²	Fruits/ m ²	Fruits infected %	Infected fruits/ m ²	Fruits/ m ²	Fruits infected (%)	Infected fruits/ m ²
33	0.13	20	3	0.47	0.63	40	25
95	0.45	16	7	0.28	56	16	0.25	36	9
97	0.08	10	1	0.90	57	52	0.25	20	5
120	0.83	17	12	0.77	0.54	38	21

^aValues based on area under canopy of nine trees per orchard.

^bEach value based on 50 fruits per orchard.

^cEach value represents the number of fruits infected with *Mucor piriformis* per square meter $\times 100$.

on 29 September 1984 was 1.5×10^5 spores per cubic centimeter of soil. The population density appeared to increase slightly in November, then decrease gradually through May 1985 (Fig. 2). The population density declined rapidly from May through July and remained between 0 and 9×10^2 sporangiospores per cubic centimeter for the duration of the experiment (Fig. 2). Population densities of sporangiospores in half-plots that were amended with pear fruits on 24 September 1986 increased to 2×10^3 and 9.6×10^3 spores per cubic centimeter in November 1986 and January 1987, respectively. Corresponding densities in unamended soil were 47 and 79 sporangiospores per cubic centimeter in November and January, respectively (Fig. 2). Soil temperature exceeded 20 C only during June, July, and August each year.

Soil amendment with pear fruits. Although the different orchard soils showed marked differences in population densities of *M. piriformis*, in all four orchards the density of sporangiospores in soil around trees receiving pear fruits was higher during at least 1 mo of the study than population densities in soil that was not amended with pear fruits (Table 1). Population densities in soils from around trees receiving no pears remained low, with little apparent seasonal fluctuation. Population densities in soil amended with pear fruits increased in October to December and remained relatively high until February to April when densities declined (Table 1).

Density and incidence of infection of pear fruits on the orchard floor. Fruit density varied considerably among orchards and seasons, e.g., orchards with the highest density of fruit on the orchard floor were 120, 97, and 33 in 1985, 1986, and 1987, respectively (Table 2). Over the 3-yr study fruit density in orchard 33 increased from 0.13 to 0.63 fruit per cubic meter, whereas fruit density in orchard 120 decreased from 0.83 to 0.54 (Table 2). The percent of fruit infected by *M. piriformis* was greatest in orchard 33 and lowest in orchard 97 in both 1985 and 1987. A comparison of fruit infection among orchards in 1986 could not be made because of incomplete data resulting from mowing of the orchard vegetation and fruit on the ground. The number of infected fruits per square meter was highest in orchards 120 and 33 in 1985 and 1987, respectively, and lowest in orchard 97 in both years (Table 2).

DISCUSSION

Natural population densities of sporangiospores of *M. piriformis* in soil in four orchards showed similar cyclic patterns over the 3-yr study. Generally, large increases occurred between October and December each year, reaching maximum levels about 1–2 mo after the increase began. Population density peaks were sharp and were followed by a rapid decline to low densities, which were maintained until the next autumn. Population density increases consistently occurred a few months after harvest when fallen fruits were abundant on the orchard floor, and cool, early winter weather had begun.

Although optimum temperature for growth of *M. piriformis* is 20 C (1), it grows and sporulates extensively at lower temperatures. Fall and winter rains easily dislodge the sporangiospores produced on decayed fruit and wash them into the soil, thus, the large increase observed in soil that was amended with pear fruits (Fig. 2 and Table 1). In contrast, relatively few sporangiospores of *M. piriformis* were observed in soils kept free of fruits.

Although the fungus sporulated on fallen leaves and soil near decaying pear fruits during November, sporangiospore population densities in soil amended with partially decomposed leaves did not increase. In previous laboratory experiments (6), sporangiospores of *M. piriformis* germinated, grew, and sporulated on fresh leaves of peach, ryegrass, and chickweed but not in nonautoclaved soil amended with leaf pieces. *M. piriformis* was a poor competitor compared with other soil saprophytes (6).

In controlled experiments, fruits were an important nutrient base for the buildup of *M. piriformis* in soil, but this relationship was less clear in commercial orchards. The percentage of infected fruit on the orchard floor sometimes was related to population densities of sporangiospores in the soil at harvest. For example, in September 1985, soil population densities of 371, 391, 811, and 915 sporangiospores per square centimeter in orchards 97, 95, 120, and 33, respectively, corresponded to 10, 16, 17, and 20% infected fruits (Fig. 1 and Table 2). Similarly, in November 1987, soil populations of 114 and 13 sporangiospores per cubic centimeter in orchards 33 and 97, respectively, corresponded to 40 and 20% fruit infection with *M. piriformis*. However, in September through November 1986, the population density of *M. piriformis* in soil in orchard 95 was between 0 and 59 per cubic centimeter, whereas fruit infection was 56%. Apparently, infection of fruit on the orchard floor was related to factors other than the population density of sporangiospores in the soil. Previously, we showed that insects and rodents were involved in rapid fruit-to-fruit spread of *M. piriformis* (7). Recently, Michailides (*unpublished*) found that additional vectors of *M. piriformis* include vinegar flies (*Drosophila melanogaster*) and nitidulid beetles (*Carpophilus hemipterus* and *C. freemani*).

Density of infected fruit on the orchard floor appears to be an important factor affecting the buildup and the maximum population densities of *M. piriformis* in orchard soil. This relationship was established clearly in controlled experiments, but only with extreme (fruit versus no fruit) treatments (Fig. 2 and Table 1), and was less obvious in commercial orchards. For example, in 1985, orchard 120 had the highest density of fruit infected by *M. piriformis* (0.12/m²) and also the highest maximum pathogen population density (1.3 × 10³/cm³) of all orchards in succeeding months (Fig. 1 and Table 2). However, this relationship did not occur in 1986 or 1987, and additional factors probably were involved. For example, sporangiospores of *M. piriformis* are clustered in both the horizontal and vertical planes in soil (3,10), and spread of sporangiospores from an infected fruit may be very limited (3). Thus, a high density of infected fruit may result in high population densities of sporangiospores in soil only within a few centimeters of the fruit, and proper sampling design and frequency are extremely important to establish correct population densities. Wind dispersal is not a means for spread,

because sporangiospores of *M. piriformis* are embedded in a mucilaginous matrix (7). Thus, other factors are important for spore dispersal and for a more uniform increase of *M. piriformis* in soil throughout the orchard. For example, in October 1986 in orchard 33, the orchard floor was mowed and fruits were chopped and scattered. The small scattered fruit pieces infected with *M. piriformis* may have resulted in the high population densities (2.6–7.3 × 10³/cm³) measured in this orchard in December 1986 and January 1987 (Fig. 1). In a similar situation, the population densities of *M. piriformis* in an orchard soil increased sharply in December 1987 after heavy feeding by a flock of birds, resulting in scatter of infected pieces of pear fruits through the orchard (R. A. Spotts, *personal observation*).

Increases of *M. piriformis* in soil did not occur, even near infected fruits, during the summer or within a few weeks after harvest. Soil temperature during these months remained above the optimum for growth of *M. piriformis* (5,11). *M. piriformis* cannot compete effectively for nutrients with other soil microbes at temperatures above 20 C (Dobson and Spotts, *unpublished*). Thus, for *M. piriformis* to increase in soil, both a favorable nutrient status and temperature are required. Additional studies are under way in our laboratory on the quantitative relationships between population densities of sporangiospores of *M. piriformis* in soil with soil temperature and soil moisture.

We conclude that the significant increases of fungal propagules of *M. piriformis* after harvest are related directly to inoculum produced by rotting fruits on the orchard floor. Orchard sanitation practices, such as minimizing fallen fruit or removing it from the orchard, could eliminate the cyclic patterns (increase-decrease) of inoculum observed in pear orchards in the Hood River Valley of Oregon. In addition, such suppression of the buildup of propagules in soil may result in less decay in stored pear fruits, since orchard soil attached to harvest bins is the primary source for contamination of pears during the dump-tank operation (7).

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