Distribution of Sporangiospores of *Mucor piriformis* in Pear Orchard Soils

R. L. DOBSON, Postdoctoral Research Associate, and R. A. SPOTTS, Professor, Department of Botany and Plant Pathology, Oregon State University, Mid-Columbia Agricultural Research and Extension Center, Hood River, OR 97031

ABSTRACT

Dobson, R. L., and Spotts, R. A. 1988. Distribution of sporangiospores of *Mucor piriformis* in pear orchard soils. Plant Disease 72:702-705.

Sporangiospores of *Mucor piriformis* were found to have a clustered spatial distribution in both vertical and horizontal planes of the soil in pear orchards. Between 76 and 93% of the spores detected were found in the top 2 cm of the soil profile, and spore numbers decreased rapidly with increasing depth. In the horizontal plane, the frequency count data for sporangiospores were described with a negative binomial model. This clustered spatial pattern reflects the colonization of a limited substrate with an irregular distribution as well as a limited spore dispersal.

Mucor piriformis Fischer is a post-harvest rot pathogen of pears (*Pyrus communis* L.) (2). This fungus is soilborne and enters packinghouse dump tank water when soil adheres to the bins and is removed during immersion of bins (12). Although chlorine is added to the dump tank water to help reduce the number of viable pathogenic propagules, there are no registered fungicides that effectively control decay of apple and pear caused by *M. piriformis* (Spotts, *unpublished*) Thus, alternate strategies of control are needed. Development of these strategies will require a better understanding of the biology of *M. piriformis*, including how the organism is distributed in soil.

Bertrand and Saulie-Carter (2) surveyed the top 5 cm of orchard soils in Hood River Valley and recovered *M. piriformis* in 21.4% of the orchards. Spotts and Cervantes (17) sampled soils in 97 orchards in the Hood River Valley at 0–5, 5–15, and 15–30 cm. Over 75% of the sporangiospores detected were found in the top 0–5 cm of the soil profile. At this soil depth, some orchard soils were found to contain no sporangiospores while others had populations of over 2,000/gm of dry soil. Correlations with several physical and chemical soil parameters at 0- to 5-cm depths did not satisfactorily explain the differences in population levels. Recently, Sholberg and Owen (14) sampled the top 5 cm of soil beneath pear tree canopies in the Okanagan Valley of British Columbia and found *Mucor* spp. in 49 of 51 orchards. Distribution of propagules in the soil was not studied.

Spatial distribution of other soilborne fungal plant pathogens in field soils is seldom random or regular (16) and is often described as clustered (1,4,6,8,15). These patterns can be observed in both vertical and horizontal planes (1). *Rhizoctonia solani* Kühn, for example, was shown to be clustered in the top 10 cm of the soil profile (13) and clustered in the horizontal plane of the field based on symptoms (3) and propagule number frequencies (8).

Spatial patterns have important implications on sampling techniques and in the interpretation of observations. In one study, random samples did not give accurate population estimates of a clustered distribution of inoculum in soil (6). Consequently, the present study was initiated to obtain more detailed information on vertical and horizontal distribution of sporangiospores of *M. piriformis* in soil.

MATERIALS AND METHODS

Vertical distribution. To study spore distribution in the soil profile, 2 ml of sterile, distilled water containing 7.5×10^5 sporangiospores per milliliter of *M. piriformis* from 3-day-old cultures grown on acidified potato-dextrose agar (PDA) were applied to an approximately 1-cm^2 area of the soil surface in a pear orchard at the Mid-Columbia Agricultural Research and Extension Center (MCAREC), Hood River, OR. The same area was then sprayed with 50 ml of distilled water. Infestations were done in February and were repeated at two locations in the orchard. Sites received 1.5–2.0 and 8.7–21.0 cm of rain before being assayed after 2 and 30 days, respectively. After 2 and 30 days, three soil cores were taken with a soil probe (Oakfield Apparatus Co., Oakfield, WI) from each of the two infested sites, and the population of *M. piriformis* was determined at 0- to 10-cm depths. Cores 2 cm in diameter were brought into the laboratory intact and cut into sections 2 cm long. A 0.5-cm-long section of soil at the 4-cm depth removed for moisture determination was weighed, oven-dried for 24 hr at 30 C, and reweighed. Dried soil weights were converted to soil volumes based on a bulk density of 1.10 as determined by the OSU Soil Physics Laboratory, Corvallis.

Each 2-cm section of soil was placed in 10 ml of distilled water and mixed for 1 min with a vortex mixer. Then, four 0.1-ml aliquots were spread, using a sterilized glass rod, onto acidified PDA (0.75 ml of acetic acid/500 ml of Difco PDA). The number of colonies of *M. piriformis* on each plate was counted after incubation at 5 C for 3–4 days. Variation of counts among plates within samples was small, and populations were expressed as average number of propagules per cubic centimeter of soil. At 5 C, *M. piriformis* sporangiospores germinate and grow faster than most other soil fungi, facilitating identification.

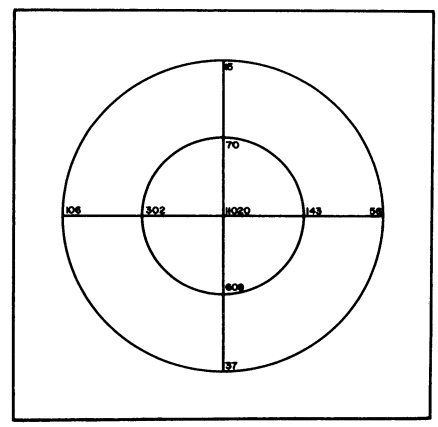

Fig. 1. Horizontal distribution in the top 2.5 cm of soil of sporangiospores of *Mucor piriformis*. Infected pear was placed at the center of the sampling area. Each value represents the number of sporangiospores per cubic centimeter and is the mean of three replicate cores from four repeated experiments. Sampling points on the circle radius are 10 cm apart.

This project was supported in part by cooperative agreement 58-9AHZ-2-109 between the USDA and Oregon State University for pome fruit disease research and by the Winter Pear Control Committee.

Use of trade names does not imply endorsement by Oregon State University of the products named or criticism of similar products not mentioned.

Oregon Agricultural Experiment Station Technical Paper 8364.

Accepted for publication 15 March 1988 (submitted for electronic processing).

To study the natural distribution of sporangiospores, soil cores, taken to an 8-cm depth, were collected at four sites in each of four orchards near Hood River. Cores were collected between early February and late March 1986. The orchards were coded 33 (Parkdale), 95 and 97 (Westside), and 120 (Eastside) to agree with orchard designations in previous studies (12,17). The 8-cm-long soil cores were divided into four sections, and the population of *M. piriformis* in each 2-cm core section was determined as described above. Dried soil weights were converted to soil volumes based on bulk densities of 0.77, 1.11, 1.07, and 1.00 for orchards 33, 95, 97, and 120, respectively, and populations were expressed as number of propagules per cubic centimeter of soil. Relationships between spore populations and soil depth for both infested and natural soils were described with linear regression after transforming spore counts to $\log_e$.

Horizontal distribution. A pear fruit infected with *M. piriformis* was placed on the soil surface in each of four locations in an orchard at the MCAREC. Fruits were covered with galvanized wire with 0.5-cm^2 openings to prevent feeding by birds and rodents. A 10-g soil sample was collected at each site for soil water determination. Three soil samples 11 mm in diameter from the top 2.5 cm of soil were collected after 1 mo at the center and at 10- and 20-cm distances in each of four directions (north, east, west, and south) from the inoculated site (Fig. 1). Samples were weighed, mixed in 10 ml of distilled water, and plated as described previously. The number of *M. piriformis* colonies was determined after 3–4 days at 5 C.

To determine natural horizontal distribution of sporangiospores, two sampling sites were selected arbitrarily within a row of pear trees and spore populations in the top 2.5 cm of soil were determined using the same procedure as in the previous experiment. Three soil samples were taken at 10-cm distances up to 50 cm in four directions from the center of the site. Samples were collected on 8 May 1986. Because the variance of the population exceeded the mean, a negative binomial model was selected to describe the distribution of sporangiospores (5). The chi-square test was used to evaluate the model for goodness of fit.

RESULTS

Vertical distribution. When spores were applied to the soil surface, followed by drenching with distilled water or natural rainfall, over 90% of the spores that were detected were found in the top 2 cm of soil and populations decreased rapidly as soil depth increased (Table 1). Relationships between spore population and depth were highly significant at both locations at 2 and 30 days after infestation of soil. No consistent change in slope was observed, but intercepts decreased between the 2-day and the 30-day sample, indicating a similar decrease in propagule number at each depth over time. Number of sporangiospores in the top 2 cm of soil decreased 85% between 2 and 30 days after inoculation, and similar decreases occurred at other depths.

In the orchard soils, 76–93% of the sporangiospores were found in the top 2 cm of the soil profile (Table 2). The natural vertical distribution of sporangiospores was best described with linear regression models when populations were transformed to $\log_e$. Relationship between spore populations and soil depth were significant ($P = 0.05$) for three of the four orchards (Table 2). The number of sporangiospores in the top 2 cm of soil was highly variable among orchards.

Horizontal distribution. The horizontal distribution showed a high spore concentration at the site of inoculation, with lower populations 10 cm from an infected pear (Fig. 1). At the pear, the average number of sporangiospores was 11,020/cm^3 of soil, whereas at the 10-cm distance the number of spores ranged from 70 to 609/cm^3. These values were highly variable at each location; the average of the four locations is shown in Figure 1.

The natural distribution of sporangiospores in the top 2.5 cm of orchard soil was highly variable over short distances. Each sampling location presented a different spatial distribution of inoculum (Fig. 2). At site 1, sporangiospores numbered as high as 5,514/cm^3 of soil, whereas 20 cm from this point the number was 63/cm^3 of soil. At site 2 (numbers underlined in Fig. 2), sporangiospores varied from 27 to 1,870/cm^3. Average propagule densities at sites 1 and 2 were 493 and 515/cm^3, respectively. A negative binomial model was used to describe the horizontal distribution of sporangiospores, and agreement of the model with the natural spore distribution was accepted at both locations based on the chi-square test for goodness of fit ($P = 0.05$). The dispersion parameter k of the negative binomial was calculated by proportion after estimation from the maximum likelihood equation (5) and was 0.0798 at one location and 0.2115 at the other, which fits a clustered distribution pattern indicated by k values less than 2.0 (16).

Table 1. Vertical distribution of sporangiospores of *Mucor piriformis* in pear orchard soil 2 and 30 days after infestation of the soil surface with a spore suspension

	Sporangiospores/cm^3 of soil[a]			
	Site 1		Site 2	
Soil depth (cm)	After 2 days	After 30 days	After 2 days	After 30 days
0–2	42,975	7,722	77,179	9,620
2–4	8,629	402	2,867	188
4–6	758	143	409	62
6–8	150	17	38	3
8–10	28	2	0	0
Intercept[b]	11.611	9.485	12.480	9.539
Slope[b]	−0.932	−0.941	−1.340	−1.110
r[c]	0.997	0.991	0.994	0.982

[a] Each number is the average of three replications.
[b] Linear regression based on the formula $ln(y + 1) = m + bx$, where y = spore population in number per cubic centimeter and x = soil depth in centimeters.
[c] Correlation coefficient; all regressions significant at $P = 0.01$.

Table 2. Vertical distribution of sporangiospores of *Mucor piriformis* in naturally infested soils of four pear orchards[a] in Hood River, Oregon

	Sporangiospores/cm^3 of soil[b]			
Soil depth (cm)	No. 33	No. 120	No. 95	No. 97
0–2	2,823	1,579	367	399
2–4	126	154	63	23
4–6	61	24	42	13
6–8	29	7	10	10
Intercept[c]	7.956	8.005	6.264	5.824
Slope[c]	−0.723	−0.906	−0.561	−0.581
r[d]	0.928*	0.991**	0.976**	0.885[e]

[a] Orchards were designated by arbitrary numbers in a previous survey.
[b] Each number is the average of four replications.
[c] Linear regression based on the formula $ln\, y = m + bx$, where y = spore population in number per cubic centimeter and x = soil depth in centimeters.
[d] Correlation coefficient; * = $P = 0.05$, ** = $P = 0.01$.
[e] Not significant.

DISCUSSION

The distribution of sporangiospores of *M. piriformis* in orchard soils was found to have a strong clustered pattern in the vertical and horizontal planes. In the vertical plane, most sporangiospores (76–93%) were found in the top 2 cm of soil (Table 2). Most orchards in the Hood River area are not tilled, but in a few that were tilled, the sporangiospores appeared to be more evenly distributed throughout the soil than in the nontilled orchards (Dobson, *unpublished*).

M. piriformis can colonize and sporulate on organic matter (11), most of which is located in the top layers of the soil. In noncultivated orchards, the vertical distribution of the sporangiospores is a function of their movement in the soil profile and of their longevity at different soil depths. By adding inoculum to the soil surface, we found that the sporangiospores showed the same vertical gradient of propagule density as in natural soils. Thus, it appears that the vast majority of sporangiospores did not move to soil depths greater than 2 cm after 1 mo of field conditions, presumably because of retention by soil particles. If limited lateral spore movement occurs, a cone-shaped pattern would result with an apex of highest concentration at the soil surface. There was over 80% reduction in the number of sporangiospores recovered at 30 days after infestation, reflecting their short life span under the conditions of this study. In a previous study, viability of sporangiospores of *M. piriformis* declined in an exponential fashion, and loss of viability was greater at higher temperatures (10).

The horizontal distribution of sporangiospores was highly variable in the top 2.5 cm of soil (Fig. 2). This extreme variability reflected a clustered distribution of sporangiospores of *M. piriformis*. The large variance to mean ratios (2.9 and 4.2) and the small k parameter of the negative binomial (0.2115 and 0.0798) are additional indications of a clustered spatial pattern (5,16). Several authors (1,13,18) have attributed this pattern to a limited substrate distribution and limited spore dispersal. Specific organic matter such as fallen pears on the orchard floor represents a limited substrate distribution. The spatial distribution resulting from inoculation with an infected pear (Fig. 1) resembles that found in the orchard, indicating that this pattern could indeed arise from *M. piriformis* colonizing fallen pears or specific organic substrates. Two months after harvest, 23–50% of fallen pears were colonized by *M. piriformis* (12).

Sporangiospores of *M. piriformis* are dispersed in orchards by rain splash and by animals, e.g., birds, mice, and insects. Birds were observed feeding on rotten pears with actively sporulating colonies of *M. piriformis*, and pieces of these pears could be found scattered about (12). Bird and rodent feeding was prevented in the experiment illustrated in Figure 1 by covering infected pears with a wire cage, and very little horizontal spread was observed. In addition, sporangiospres of *M. piriformis* are embedded in a mucilaginous matrix (9) that firmly cements the spores to one another when dry (7) and are not known to be airborne.

The magnitude of populations within the clustered patterns is probably a function of the size of the substrate and the extent of substrate colonization and sporulation. Thus, the pattern might be very uneven, as shown in Figures 1 and 2, with sites with extremely high populations (from colonization of a large pear) to sites with lower populations (from colonization of pieces of pear). The significance of this clustered pattern over the orchard surface becomes important in sampling techniques and interpretation of the sampling results from an orchard. In one orchard we sampled, a soil core from a high population cluster composited with nine cores from lower populations changed the overall sporangiospore population of the orchard from 22 to 4,400/cm^3 of soil. Sampling methods for *M. piriformis* in orchard soils must include consideration of proximity of each sample to fallen fruit and avoid the errors that could result when samples of vastly different populations are composited.

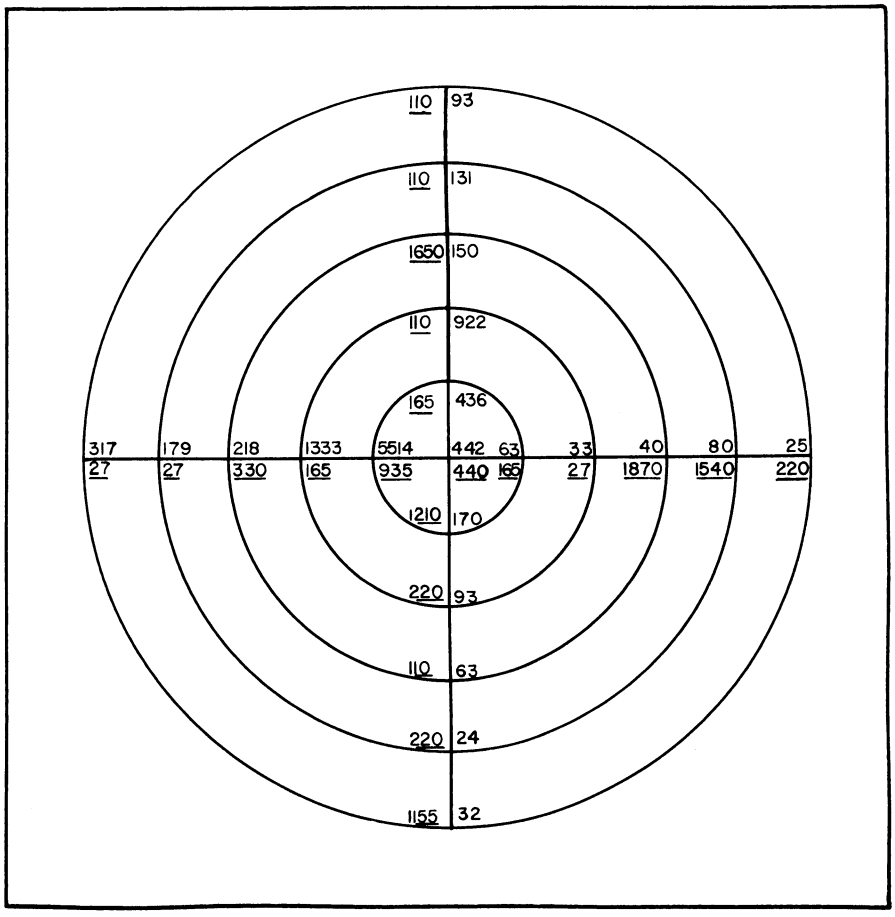

Fig. 2. Horizontal distribution at two sites in the top 2.5 cm of soil of sporangiospores of *Mucor piriformis*. Each value represents the number of sporangiospores per cubic centimeter of naturally infested soil and is the mean of three replicate cores. Values for site 2 are underlined. Sampling points on the circle radius are 10 cm apart.

LITERATURE CITED

1. Adams, P. B. 1986. Production of sclerotia of *Sclerotinia minor* on lettuce in the field and their distribution in soil after disking. Plant Dis. 70:1043-1046.
2. Bertrand, P., and Saulie-Carter, J. 1980. Mucor rot of pears and apples. Oreg. State Univ. Agric. Exp. Stn. Spec. Rep. 568. 22 pp.
3. Campbell, C. L., and Pennypacker, S. P. 1980. Distribution of hypocotyl rot in snapbean caused by *Rhizoctonia solani*. Phytopathology 70:521-525.
4. Crowe, F. J., Hall, D. H., Greathead, A. S., and Baghott, K. G. 1980. Inoculum density of *Sclerotinia cepivorum* and the incidence of white rot of onion and garlic. Phytopathology 70:64-69.
5. Elliott, J. M. 1977. Some Methods for the Statistical Analysis of Benthic Invertebrates. 2nd ed. Sci. Publ. 25. Freshwater Biological Association, Ambleside, Cumbria, England. 156 pp.
6. Hau, F. C., Campbell, C. L., and Beute, M. K. 1982. Inoculum distribution and sampling methods for *Cylindrocladium crotalariae* in a peanut field. Plant Dis. 66:568-571.
7. Ingold, C. T. 1971. Fungal Spores: Their Liberation and Dispersal. Oxford University

Press, London. 302 pp.
8. Martin, S. B., Campbell, C. L., and Lucas, L. T. 1983. Horizontal distribution and characterization of *Rhizoctonia* spp. in tall fescue turf. Phytopathology 73:1064-1068.
9. Michailides, T. J. 1980. Studies on postharvest decay of stone fruit caused by *Mucor* species. M.S. thesis. University of California, Davis. 63 pp.
10. Michailides, T. J., and Ogawa, J. M. 1987. Effect of soil temperature and moisture on the survival of *Mucor piriformis*. Phytopathology 77:251-256.
11. Michailides, T. J., and Ogawa, J. M. 1987. Colonization, sporulation, and persistence of *Mucor piriformis* in unamended and amended orchard soils. Phytopathology 77:257-261.
12. Michailides, T. J., and Spotts, R. A. 1986. Factors affecting dispersal of *Mucor piriformis* in pear orchards and into the packinghouse. Plant Dis. 70:1060-1063.
13. Papavizas, G. C., Adams, P. B., Lumsden, R. D., Lewis, J. A., Dow, R. L., Ayers, W. A., and Kantzas, J. G. 1975. Ecology and epidemiology of *Rhizoctonia solani* in field soil. Phytopathology 65:871-877.
14. Sholberg, P. L., and Owen, G. R. 1987. Incidence of pathogenic *Mucor* spp. in Anjou pear orchard soils in the Okanagan Valley of British Columbia. Can. Plant Dis. Surv. 67:9-10.
15. Smith, V. L., and Rowe, R. C. 1984. Characteristics and distribution of propagules of *Verticillium dahliae* in Ohio potato field soils and assessment of two assay methods. Phytopathology 74:553-556.
16. Southwood, T. R. E. 1978. Ecological Methods. Champman & Hall, London. 524 pp.
17. Spotts, R. A., and Cervantes, L. A. 1986. Populations of *Mucor piriformis* in soil of pear orchards in the Hood River Valley of Oregon. Plant Dis. 70:935-937.
18. Stanghellini, M. E., von Bretzel, P., Kronland, W. C., and Jenkins, A. D. 1982. Inoculum densities of *Pythium aphanidermatum* in soils of irrigated sugar beet fields in Arizona. Phytopathology 72:935-937.