Colonization, Sporulation, and Persistence of *Mucor piriformis* in Unamended and Amended Orchard Soils

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ABSTRACT

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The behavior of two isolates [California isolate (CA), Chile isolate (CH)] of *Mucor piriformis* was studied in orchard soils that were autoclaved or nonautoclaved and amended with a suspension of soil microbes, organic matter, or used without amendments. In nonautoclaved or autoclaved soils amended with soil microorganisms, *M. piriformis* failed to colonize the soil. However, in soils amended with organic matter and soil microorganisms the fungus grew and sporulated. Nonautoclaved leaves of peach (*Prunus persica*), ryegrass (*Lolium perenne*), and chickweed (*Stellaria media*) also supported sporangiospore germination, growth, and sporulation by *M. piriformis*. Sporangiospores of *M. piriformis* did not germinate and grow in nonautoclaved soil; in contrast, they germinated and grew in autoclaved soil amended or unamended with crushed peach leaves. When soil microorganisms were added to autoclaved soil, growth of *M. piriformis* was restricted to the immediate vicinity of the spore inoculum. Glucose amendments at levels of 7.5–300 μg per gram of dry soil favored sporangiospore germination in autoclaved but not in nonautoclaved soil. Germ tubes of sporangiospores that germinated in soil had unusual septation, fragmentation, and retraction of their protoplast and lysed after 15 days in soil.

Additional key words: postharvest pathogens, saprophyte.

Mucor piriformis Fischer is a postharvest pathogen that infects fruits of pears and apples (2,11), strawberries (6), and, less frequently, peaches and nectarines (12,13,15). It has been isolated from soils from regions in Europe, Canada, and the United States (5).

Members of Mucoraceae are known to survive saprophytically in the soil (19), where they play an important role in the early colonization of plant materials by using readily available sugars, and hence are referred to as sugar fungi (3). Hudson (9) reported that *Mucor hiemalis* Wehmer sporulated within 2 days after colonization of a variety of substrates. Warcup (18) showed that all colonies of *Mucor* spp. originated from sporangiospores and none from hyphae, indicating that sporangiospores were the long-term surviving propagules. Recently, we also showed that sporangiospores were the long-term surviving propagules of *M. piriformis* (14). Sporangiospores survived for at least 1 yr in unamended orchard soils, whereas mycelia survived from 7 to 40 days in natural soil. This investigation was undertaken to study saprophytic growth and spore germination of *M. piriformis* in unamended and autoclaved soils and in amended soils.

MATERIALS AND METHODS

Isolates. Two isolates of *M. piriformis* were used in this study: One was isolated from a decayed peach in California, designated CA (ATCC 52555), and the other from a decayed nectarine from Chile, designated CH (ATCC 52554). These isolates had cultural and morphological characteristics consistent with those reported for *M. piriformis* (13).

Soil colonization. Two soil types, a Reiff loam (20–25% clay, 40–45% silt, and 30–40% sand) from Davis and a Hanford fine sandy loam (14% clay, 25% silt, and 61% sand) from Parlier, were collected from the upper 10-cm soil layer in two peach orchards, and screened through a 2-mm-mesh sieve. The soils were air-dried for 3–4 days at room temperature (22 ± 1 C). A portion of each soil was autoclaved (90 min at 121 C) three times at 2-day intervals. Fifty grams of soil was placed in a glass petri plate (10 × 1.5 cm), and moistened to 20 g of water per 100 g of dry soil (−0.3 and −0.5 bar matric potential for the Reiff loam and Hanford fine sandy loam soil, respectively). A 5-mm-diameter mycelial disk, cut from the edge of a 3-day-old fungal colony growing on potato-dextrose agar (PDA) acidified (pH 3.5 ± 0.1) with lactic acid, was transferred into the soil, i.e., in a hole 5–6 mm diameter and 3 mm deep. Plates were sealed in double plastic bags and incubated at 21 C, which is the optimum temperature for growth of *M. piriformis* (12). At indicated intervals (Fig. 1), five soil samples were taken, using a cork borer (inner diameter of 5 mm), at distances of 0.5, 1, 2, 3, and 4 cm from the mycelial disk, plated on acidified PDA plates, and incubated at 0 C for 8 days in darkness. Each treatment was assayed in triplicate per sampling date.

Colonization in soil amended with soil microflora. Soil extract (suspension of microflora) was prepared by mixing 10 g of dry soil with 90 ml of sterile distilled water for 2 min (18,500 rpm) in a Waring commercial blender (Blender 5010 S, Waring Products Division, Dynamics Corporation of America, New Hanford, CT) and filtering through a four-layer cheesecloth to remove the soil. The microflora content was determined by using standard dilution plate assays with selective media, which consisted of surfactant-PDA for fungi (17), chitin water agar for actinomycetes (10), and pentachloronitrobenzene (PCNB)-soil-extract agar for bacteria (7). Five plates of each selective medium were used. Ten milliliters of this soil extract, containing a total of 20×10^6 colony-forming units (cfu) consisting of a mixture of fungi, actinomycetes, and bacteria per milliliter, were poured in petri plates containing 50 g of autoclaved dry soil and mixed thoroughly with a spatula. The plates were sealed in double plastic bags and incubated at 21 C for 25 days to allow reestablishment of the microflora. Then, a mycelial disk of *M. piriformis* was transferred into the center of each plate and reincubated at 21 C. Colonization of soil by *M. piriformis* was determined as described above.

Colonization in soil amended with natural organic substrates. Orchard soils were amended with dry, mature, green peach (*Prunus persica* (L.) Batsch. cultivar 'Elberta') leaves that were crushed, sieved to a final size of 2 mm^2, incorporated in soil at the rate of 1 g of dry leaves per 100 g of dry soil, and wetted with 20 g of

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water per 100 g of dry soil (−0.3 to −0.5 bar matric potential). Similarly, 10 ml of a peach extract [i.e., ripe peach fruits of 0.35 kg/cm^2 tissue firmness and 11.0% soluble solids (sugars [w/w]) were blended and filtered twice through Whatman No. 42 filter paper] was added to 50 g of dry autoclaved or nonautoclaved soil, which resulted in 0.36 and 0.72 g of soluble solids per 100 g of dry soil. A 5-mm-diameter mycelial disk obtained from a 3-day-old culture of *M. piriformis* grown at 21 C on acidified PDA was transferred to the center of each plate containing 50 g of amended soil. Sporulation on the surface of the autoclaved soil and mycelial growth within the nonautoclaved soil were recorded as previously described. A three-way factorial analysis of variance (ANOVA) to describe recovery of *M. piriformis* as a function of isolate, time, and distance was done with the Statistical Analysis Systems (SAS Institute, Inc., Cary, NC).

Growth of *M. piriformis* on leaves. To determine the saprophytic development of *M. piriformis* in the absence of soil, 0.5 g of dry, mature, green leaves (crushed and sieved to 2 mm^2 in size) of peach or weeds (such as ryegrass (*Lolium perenne* L.) and chickweed (*Stellaria media* L.)) obtained from a peach orchard were placed in the center of petri plates (10 × 2.5 cm) on a Whatman No. 1 filter paper moistened with 3 ml of sterile distilled water. A 5-mm-diameter mycelial disk of *M. piriformis* was then transferred to the center of each plate. Control plates with only moistened filter paper and a mycelial disk of *M. piriformis* were included.

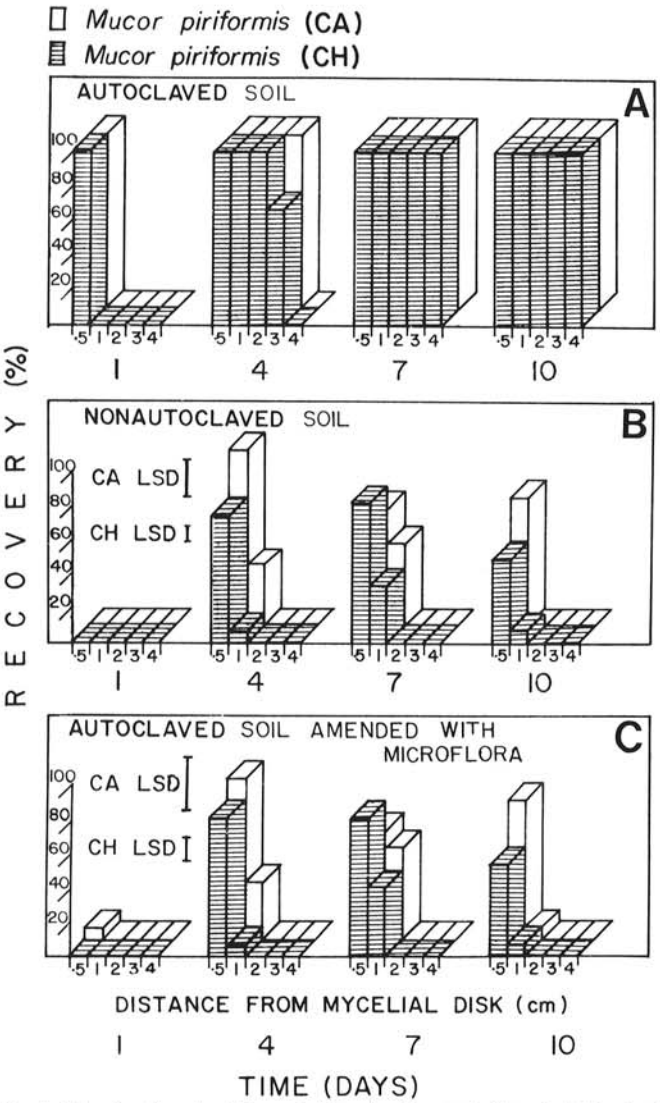

Fig. 1. Colonization of soil in petri plates by *Mucor piriformis*. A Hanford fine sandy loam soil from Parlier was adjusted to 20 g of water per 100 g of dry soil (−0.5 bar matric potential) and a 5-mm-diameter mycelial disk obtained from a 3-day-old culture of *M. piriformis* was transferred to the center of the plate before incubation at 21 C for 10 days in darkness. **A,** Autoclaved soil; **B,** Nonautoclaved (natural) soil; and **C,** Autoclaved soil amended with soil microflora (4.0× 10^6 cfu, fungi/actinomycetes/bacteria per gram of dry soil). Percent recovery was determined as the average percentage of positive isolations from five soil cores at each distance of sampling for each of three replicate plates per sampling date. Experiment was repeated twice. For LSD $P = 0.05$.

Behavior of sporangiospores in soil and leaves. Petri plates containing autoclaved and nonautoclaved soils with and without dried peach leaves were infested with 50 μl of a sporangiospore suspension (5× 10^5 spores per milliliter) obtained from a 4-day-old culture of *M. piriformis*. The plates with autoclaved and nonautoclaved soil were incubated at 21 C for 8 days and 2 mo, respectively. Sporulation on the soil surface was recorded for the autoclaved soil and colonization of nonautoclaved soil was determined by sampling soil cores at 2, 4, 6, and 8 days and after 1 and 2 mo.

To determine sporangiospore germination and colonization on dried leaf fragments, 0.5 g of peach, ryegrass, or chickweed leaves were placed in the center of petri plates on a moistened Whatman No. 1 filter paper. A 100-μl sporangiospore suspension (2 × 10^5 spores per milliliter) of *M. piriformis* was placed on the leaf fragments in the center of the plates. Plates prepared similarly but without leaves served as controls.

Behavior of sporangiospores also was tested in autoclaved, nonautoclaved, or autoclaved soil amended with a soil microflora suspension (4.0× 10^6 cfu per gram of dry soil). A sporangiospore suspension of *M. piriformis* containing 2× 10^5 spores per milliliter was mixed with molten water agar (cooled to 42–44 C) and allowed to solidify onto sterile microscope slides, which were dipped into the mixture. The slides were then buried in 500 g of soil, which was adjusted at 20 g of water per 100 g of dry soil (−0.3 to −0.5 bar matric potential) and sealed in double plastic bags. At regular intervals four slides per treatment were removed, washed with tap water, and sporangiospore germination observed with a compound microscope (×320). One hundred randomly selected sporangiospores were counted on each slide.

In another experiment, the effect of glucose amendments (of 7.5, 15, 30, and 300 μg of glucose per gram of dry soil) on sporangiospore germination was determined in autoclaved and nonautoclaved soil. Again, slides with thin-layer water agar impregnated with sporangiospores were buried in soil and germination was determined after 24 hr of incubation.

Data were analyzed with a three-way factorial analysis of variance (ANOVA). Factors included soil treatment, isolate, and

TABLE 1. Growth and sporulation of *Mucor piriformis* [California isolate (CA), Chile isolate (CH)] on the surface of autoclaved soil (Hanford fine sandy loam) with 20 g of water per 100 g of dry soil (−0.5 bar matric potential) in petri plates[a] incubated at 21 C for 10 days

Incubation time (days)	Diameter of area with sporangiophores and sporangia (cm)[b]			
	Amended soil[c]		Unamended soil	
	CA	CH	CA	CH
2	2.8	2.3	—[d]	—[d]
3	5.0	4.1	2.4	1.9
4	7.7	5.4	3.8	3.2
5	9.0	7.0	4.9	4.2
6		7.7	5.7	5.1
8		9.0	9.0	7.4[e]

[a] 50 μl of a 5× 10^5 spore per milliliter suspension obtained from a 4-day-old culture of *M. piriformis* were transferred to the center of the petri plates.
[b] Diameter of sporulation area is the average from three replicate plates. ANOVA was done with the Statistical Analysis Systems (SAS Institute, Inc., Cary, NC). This analysis indicated that the diameter of the area colonized by the isolate CA was significantly larger than that of the area colonized by the isolate CH ($F = 297.4$; df = 1; $P = 0.01$); and colonization of amended soil by either isolate was faster than of the unamended soil ($F = 1{,}197.4$; df = 1; $P = 0.01$).
[c] Soil was amended with 1 g of crushed peach leaves per 100 g of dry soil.
[d] Sporulation was evident only on the infestation sites.
[e] Surface of the soil was covered with sporulation to the edges of the petri plates after 9–10 days.

time (Tables 1 and 2) or soil treatment, isolate, and glucose concentration (Table 3).

RESULTS

Soil colonization. The data for the two different soils were similar ($P > 0.05$) and therefore results for only one soil are reported. In autoclaved soil, both fungal isolates colonized the Hanford fine sandy loam soil from Parlier to a distance of 4 cm from the mycelial disk after 7 days (Fig. 1A). Abundant sporangia were produced on the soil surface.

In nonautoclaved soil, mycelial growth was limited to 0.5–1-cm distance from the mycelial disk after 10 days (Fig. 1B). Although sporulation was evident on the mycelial disks, no sporulation occurred on the soil.

Soils amended with soil microflora. In autoclaved soil amended with soil microflora, the growth of both isolates was comparable to that in nonautoclaved soil. Again, neither isolate was recovered beyond 1 cm from the mycelial disk after 10 days (Fig. 1C). Sporangia developed on the disks after 2 days, but none on the surface of the soil.

Colonization in soils amended with natural organic substrates. In autoclaved soils amended with peach leaves, sporangiophores and sporangia of *M. piriformis* developed at a distance of 3 cm from the mycelial disk after 3 days and covered the plates after 4 days. In nonautoclaved soil amended with crushed leaves, both isolates colonized the soil to a distance of 0.5 cm within the first day of incubation and were recovered at levels of 100 and 47% for isolate CA and CH, respectively (Fig. 2A). After 30 days, recovery at a 2-cm distance was 73% for isolate CA and 13% for isolate CH (Fig. 2A).

In nonautoclaved soil amended with 0.36 or 0.72 g of peach extract, both isolates colonized the soil to a 0.5-cm distance from the mycelial disks after 1 day with 60–100% and 35–53% recovery for isolate CA and CH, respectively (Fig. 2B and C). In general, isolates CA and CH were not recovered beyond a 1-cm distance after 15 days (Fig. 2B and C) except isolate CA, which was recovered after 30 days at a 2-cm distance from the mycelial disk in soil amended with 0.36 g of peach extract per 100 g of air-dried soil (Fig. 2B). By this time the soils were largely covered with mycelia of *Geotrichum* spp., *M. racemosus* Fres., and *Fusarium* spp.

Growth of *M. piriformis* on leaves. Crushed leaves of peach, ryegrass, and chickweed were colonized in petri plates within 1 day; sporangia were apparent after 1 day on the mycelial disks and by

TABLE 2. Sporangiospore germination of *Mucor piriformis* [California isolate (CA), Chile isolate (CH)] on water-agar slides buried in a Hanford fine sandy loam soil (of −0.5 bar matric potential) incubated at 21 C for 30 days

Time of burial (days)	Sporangiospore germination (%)[a]					
	Autoclaved soil		Nonautoclaved soil		Autoclaved soil amended with microflora[b]	
	CA	CH	CA	CH	CA	CH
1	9.0	0.5	1.0	0.0	1.0	0.3
2	4.0	2.3	0.0	0.0	0.8	0.5
3	7.0	0.8	0.0	0.0	0.8	0.5
5	3.0	0.5	0.0	0.0	0.5	0.5
10	3.5	0.8	0.0	0.0	0.5	0.8
15	3.3	0.5	0.3	0.0	0.5	0.0
20	3.3	2.3	0.0	0.0	0.5	0.5
30	6.5	0.3	0.0	0.0	0.5	0.5

[a] Percentage of germination is the average of four microscope slides; 100 sporangiospores counted per each slide. ANOVA done on the arc sine transformed data with the Statistical Analysis Systems (SAS Institute, Inc., Cary, NC). This analysis indicated that spore germination of the two isolates was significantly different ($F = 77.14$; df = 1; $P = 0.01$); germination in the differently treated soils was different ($F = 160.40$; df = 2; $P = 0.01$); and the effect of time of burial in soil on spore germination was not significant ($F = 2.15$; df = 7; $P > 0.05$).

[b] Soil amended with microflora was adjusted to 4.0×10^6 cfu of fungi/actinomycetes/bacteria per gram of dry soil.

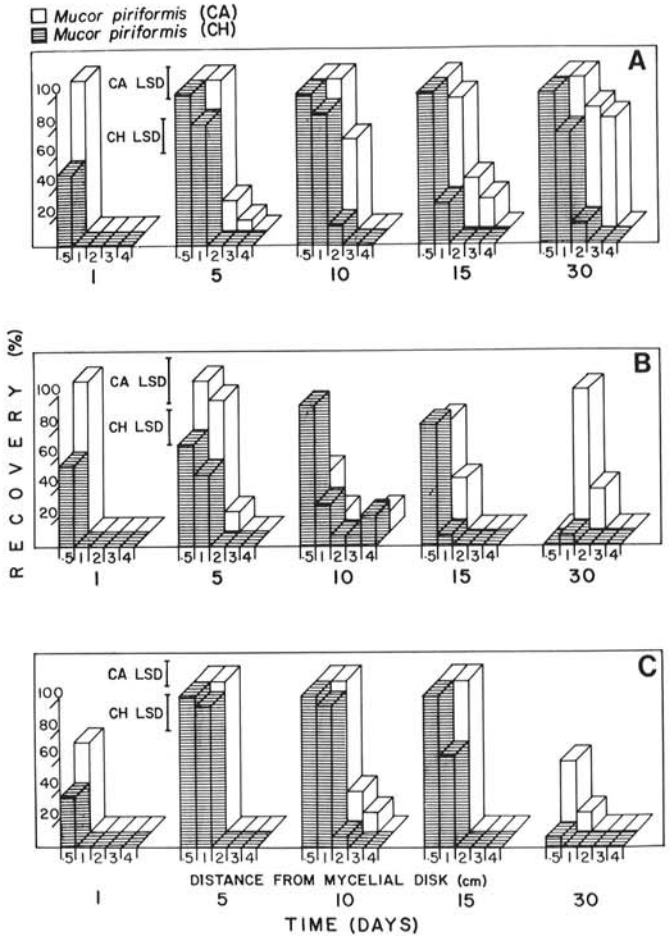

Fig. 2. Colonization of soil in petri plates by *Mucor piriformis*. A nonautoclaved Hanford fine sandy loam soil from Parlier was amended with either crushed peach leaves or peach fruit extract. A 5-mm-diameter mycelial disk that was obtained from a 3-day-old culture of *M. piriformis* was transferred to the center of the plate before incubation at 21 C for 30 days in darkness. **A,** Soil amended with 1 g of crushed, dry peach leaves per 100 g of dry soil; **B,** Soil amended with 0.36 g of soluble solids peach fruit extract per 100 g of dry soil; and **C,** Soil amended with 0.72 g of soluble solids peach fruit extract per 100 g of dry soil. All soils (A, B, and C) were adjusted at −0.5 bar matric potential by the addition of water (A) or with the respective solutions of peach extract (B and C). Percent recovery was determined as the average percentage of positive isolations from five soil cores at each distance of sampling for each of three replicate plates per sampling date. Experiment was repeated twice. For LSD $P = 0.05$.

TABLE 3. Effect of glucose amendments on the germination of sporangiospores of *Mucor piriformis* [California isolate (CA), Chile isolate (CH)] on water-agar slides buried in a Hanford fine sandy loam soil[a] from Parlier and incubated at 21 C for 24 hr in darkness

Glucose μg/g dry soil	Sporangiospore germination (%)[b]			
	Autoclaved soil		Nonautoclaved soil	
	CA	CH	CA	CH
0.0 (Nonamended)	5.0	1.0	0.0	0.0
7.5	44.5	46.3	0.0	0.0
15.0	84.5	86.3	0.3	0.0
30.0	88.5	78.5	0.0	0.0
300.0	81.5	71.8	0.0	0.0

[a] Soil was adjusted to 20 g of water per 100 g of dry soil (−0.5 bar matric potential) by adding glucose solutions or sterile water (nonamended soil).

[b] Percentage of germination is the average of four slides; 100 sporangiospores were counted per each slide. ANOVA was done on the arc sine transformed data with the Statistical Analysis Systems (SAS Institute, Inc., Cary, NC). This analysis indicated that in autoclaved soil amended with glucose spore germination of the isolate CA was greater than of the isolate CH ($F = 12.71$; df = 1; $P = 0.01$) and the effect of glucose concentration was significant ($F = 329.2$; df = 4; $P = 0.01$).

the second day on the leaves. Control plates containing only moistened Whatman No. 1 filter paper did not show any growth or sporulation of *M. piriformis* beyond the mycelial disk, indicating that any diffusion of nutrients from the disk could not support mycelial growth or sporulation.

Autoclaved soils amended with peach fruit extract were covered with fungal sporulation after 4–6 days for either isolate to a distance of 4.5 cm. Sporulation of both isolates was better in the soil amended with the 0.72 g of soluble solids extract per 100 g of dry soil.

Behavior of sporangiospores in soil and leaves. In autoclaved soil plus dried peach leaves, the sporangiospores germinated and abundant sporulation was evident after two days (Table 1). Sporulation of the CA isolate covered the plates after 5 days and that of the CH after 8 days. In autoclaved, unamended soils, sporulation of CA covered the plates after 8 days and of CH after 9–10 days (Table 1). The ANOVA indicated that in both amended and unamended soils the diameter of area colonized by the isolate CA was significantly larger than that of the area colonized by the isolate CH ($F = 297.4$; df = 1; $P = 0.01$). In addition, colonization of amended soil by either isolate was faster than that of the unamended soil ($F = 1{,}197.4$; df = 1; $P = 0.01$).

In nonautoclaved soil amended with peach leaves, sporulation of *M. piriformis* was evident about 0.3–0.5 cm beyond the infestation sites by the second day. Mycelium and sporulation of *Rhizopus stolonifer* (Ehr.:Fr.) Lind. were evident by the third day and those of *M. racemosus* and *Fusarium* spp. by the fourth and fifth day after incubation. In nonautoclaved, unamended soil, sporulation of *M. piriformis* was not detected even after 8 days of incubation. Although recovery was 7–27% for isolate CA at 0.5 and 1.0 cm after 2 days, we believe that these colonies originated from spores which, when added, were carried in the water through the soil to that distance. After 2 mo, *M. piriformis* was not recovered, even at a distance of 0.5 cm from the infestation site. The sporangiospores of the CH isolate showed a similar isolation pattern.

Sporangiospores embedded in thin-layer water agar slides (buried in autoclaved soil) germinated 3–9% and 0.3–2.3% for isolates CA and CH, respectively (Table 2). Germination of sporangiospores of isolate CA was generally significantly higher than germination of those of isolate CH ($F = 77.14$; df = 1; $P = 0.01$). After 5 days, several sporangia were noticed on the surface of the soil adjacent to the slides as well as on the slides. Some of the hyphae showed unusual septation, fragmentation, and retraction of the protoplast. After 15 days, tips of germ tubes appeared open and protoplasmic material was exuded as the hyphae lysed. Occasionally a few sporangiospores of isolate CA germinated on slides buried in nonautoclaved (natural) soil (Table 2). A very small percentage of sporangiospores from both isolates germinated on slides buried in autoclaved soil amended with soil microflora (Table 2). Sporangiospore germination was higher in autoclaved than in the autoclaved soil amended with microflora or in nonautoclaved soil ($F = 160.4$; df = 2; $P = 0.01$).

Sporangiospore germination on the slides increased from 1–5% to about 45% when autoclaved soil was amended with 7.5 μg of glucose per gram of dry soil and to 70–90% when soil was amended with 15–300 μg of glucose per gram of dry soil (Table 3). Glucose addition to nonautoclaved soil at 7.5–300 μg per gram of dry soil did not induce any sporangiospore germination (Table 3). In general, sporangiospore germination of isolate CA was higher than that of isolate CH ($F = 12.7$; df = 1; $P = 0.05$) in autoclaved soil amended with glucose. The effect of glucose concentration was significant ($F = 329.2$; df = 4; $P = 0.01$).

DISCUSSION

The results indicate that *M. piriformis* can grow saprophytically in nonautoclaved orchard soil. However, the 1–2-cm extension in mycelial growth from the mycelial disk and the lack of sporulation in natural unamended soil suggest that *M. piriformis* is a poor competitor compared with other soil saprophytes and other *Mucor* spp. Similar behavior patterns were realized when autoclaved soils were amended with a suspension of soil microflora (Fig. 1C). These results are in accordance with those of Hawker (8), who reported that *Mucor* spp. colonized rapidly and sporulated conspicuously on soil treated at 100 C but not in soil treated at 60 C, presumably because of resident antagonistic actinomycetes and bacteria (1). Fungal colonization in autoclaved soil suggests that *M. piriformis* can be established where antagonistic soil microflora have been removed.

The detection of *M. piriformis* at distances of 2–3 cm beyond the mycelial disk in natural soil amended with organic matter and the persistence of recovery even after 30 days' incubation in the soil (Fig. 2A–C) suggest that the organic amendments provided a carbon source for the test organism. These amendments supported sporangiospore germination, growth, and sporulation of *M. piriformis*. Thus, *M. piriformis* showed some competitive saprophytic ability. Hence, these results suggest that under orchard conditions the fungus is able to use nutrients derived from fallen peach leaves, fruits, and other vegetative matter. However, the competitive nature of *M. piriformis* may be short lived as mycelial growth and sporulation of other fungi such as *R. stolonifer*, *Fusarium* spp., *Penicillium* spp., *Geotrichum* spp., and *M. racemosus* were evident on amended soils. Also, extensive mycelial lysis occurred after 15 days of incubation in orchard soil and recovery of viable mycelia dropped drastically after that date (14).

Nongermination of sporangiospores of *M. piriformis* in nonautoclaved soil and a low percentage of germination in autoclaved soil and autoclaved soil amended with soil microflora (Table 2) suggest that the majority of sporangiospores are in a passive state in soil, regardless of the absence or presence of soil microflora. This inactivity assures growth of the fungus only under favorable conditions and is therefore of high survival value. Chinn and Tinline (4) reported that some spores of *Cochliobolus sativus* (Ito & Kurib.) Drechs. ex Dastur germinated immediately on contact with soil and perished, whereas those with an inherent capacity for dormancy survived for prolonged periods. Similarly, many of the sporangiospores of *M. piriformis* in soil are probably dormant (most of the spores do not germinate) and, therefore, capable of long-term survival. In fact, sporangiospores of *M. piriformis* survived for at least 1 yr in natural soil (14).

Although presence of soil microflora in autoclaved soil prevented colonization by mycelia of *M. piriformis* or germ-tube development (Fig. 1C), these natural soil microbes had some effect on the germination of sporangiospores (Table 2). The fact that the majority of sporangiospores do not germinate in natural nonamended soil or in natural glucose-amended soil but do germinate in autoclaved soil amended with glucose indicates that both availability of adequate nutrients and absence of antagonistic microflora favor sporangiospore germination (Table 3). Even though only a small percentage of sporangiospores of either isolate germinated in autoclaved soil (Table 2), colonies originating from these spores grew rapidly, colonized the soil, and covered test plates with abundant sporulation. But development of colonies of *M. piriformis* in autoclaved soil amended with soil microflora was restricted to the mycelial disk, indicating that antagonistic soil microflora, such as those added, prevented fungal activity. Sporulation of *M. piriformis* in natural soil amended with natural substrates within 2 days after infestation indicates that even the very small numbers of germ tubes that survive lysis would be capable of producing large numbers of sporangiospores. Failure of a germ tube to grow and produce sporangia in soil does not necessarily result in its lysis, because retraction of its protoplast probably provides an additional mechanism for survival. Retracted protoplasts also were observed in mycelia of *M. piriformis* buried in natural soil (14) as well as in germ tubes from sporangia of *Pythium ultimum* Trow in infested soil (16). As was suggested in that study (16), protoplast conservation by retraction enabled the fungus to survive under starvation conditions.

In conclusion, the quick sporulation of both isolates on newly occupied substrates in environments of low antagonistic microflora results in production of additional propagules for continuance of the life cycle of *M. piriformis* in soil.

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