

Effect of Soil Temperature and Moisture on the Survival of *Mucor piriformis*

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ABSTRACT

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Temperature was a more important factor than moisture in affecting survival of sporangiospores of *Mucor piriformis* [California isolate (CA), Chile isolate (CH)]. In a Reiff loam soil, sporangiospores showed longest survival at 0 and 21 C and least survival at 36 and 39 C. Sporangiospores survived better in dry (-1,300 bars matric potential) than in wet (-0.3 bar matric potential) or wet-dry soil (-0.3 to -1,300 bars matric potential) at all temperatures except 0, 36, and 39 C. Viability of propagules (mycelia and spores) of *M. piriformis* mixed with soil and buried 5 and 10 cm deep in this soil at Davis, and in a Hanford fine sandy loam at Parlier, CA, declined over time in an exponential fashion. At Davis, where weekly mean temperatures of soil were lower than 27 C, spores of both isolates survived

up to 1 yr. In contrast, at Parlier, where weekly mean temperatures of soil were higher than 27 C, the decline of propagule viability was faster and only the CA isolate buried at 10-cm depth survived for a year. Similarly, on inoculated and mummified peaches buried 5 cm deep in soil, the CA isolate was recovered after 19-20 mo at both locations and the CH isolate after 9 and 20 mo, respectively, for Parlier and Davis locations. Microscopic observation of colonies of *M. piriformis* from propagules surviving a year in soil revealed that all colonies originated from sporangiospores. Mycelia buried in soil survived 40 and 21 days under laboratory and field conditions, respectively.

Additional key words: moisture, nectarines, peaches, postharvest pathogen, soil, temperature.

Mucor piriformis Fischer is reported as a postharvest pathogen of fruit of pears, apples, strawberries, and occasionally of peaches and nectarines in Europe (7,13), Canada (20), and the United States (2,3,15,17,21,22,25). In California, this fungus was not considered a postharvest pathogen of stone fruit until 1977 when a serious outbreak of decay caused by *M. piriformis* occurred on shipments of fresh-market peaches from California to eastern U.S. markets and of nectarines from Chile to California.

In contrast to other fruit-rotting fungi, the occurrence of *M. piriformis* has been reported infrequently in the world, and its known distribution is limited to the temperate zones (10,11). However, isolations from soil have been reported in several European countries, Taiwan, Colombia, Canada, and the United States (10). The recovery of *M. piriformis* from both cultivated and uncultivated soils (4,6,18) suggests that orchard soil could be an inoculum source. Bertrand and Saulie-Carter (3) reported that there was a reservoir of *M. piriformis* in soil of pear orchards in Oregon and in dump tanks at packing houses (2,3). They suggested (3) that orchard soil and/or tank water may be the sources of fruit contamination. To our knowledge, *M. piriformis* has not been isolated from California soils. However, during 1962-1980 a survey of pathogenic fungi associated with California stone fruit showed the presence of *M. piriformis* on peaches and nectarines and *M. circinelloides* v. Tieghem, *M. racemosus* Fres., and *M. plumbeus* Bonorden on various stone fruits (21).

In three studies, the survival of sporangiospores of *M. piriformis* was determined either in glass distilled water (8), on potato-dextrose agar (PDA) media (25), or on fruit surfaces (26). However, no studies exist concerning the viability of *M. piriformis* in soil. An understanding of the survival patterns of this fungus in soil is required to forecast its importance in postharvest decay of stone fruits. The purpose of this study was to determine how *M. piriformis* is able to survive in orchard soils. We used two isolates of *M. piriformis*, which differed in sensitivity to temperature (22). A preliminary report of this work was published (23).

MATERIALS AND METHODS

Isolates. Two isolates of *M. piriformis*, one isolated from a decayed peach in California (designated CA) (ATCC 52555) and another isolated from a decayed nectarine shipped to California from Chile (designated CH) (ATCC 52554), were used.

Survival of sporangiospores in soils at various temperatures and moistures. We used soil from two peach orchards, one a Reiff loam (20-25% clay, 40-45% silt, and 30-40% sand) from the experimental field plot of the Department of Plant Pathology, University of California at Davis, and the other a Hanford fine sandy loam (14% clay, 25% silt, and 61% sand) from the University of California Kearney Agricultural Center at Parlier. The soils were screened through a 5.6-mm-mesh sieve to eliminate large debris and air-dried at 22 ± 1 C on a laboratory bench for 4-5 days. The air-dried soil (1-2 g of water per 100 g of soil) was rescreened through a 2.0-mm-mesh sieve and used without autoclaving. Percent water content at field capacity was 31.5% for the Reiff loam soil and 24% for the Hanford fine sandy loam determined with ceramic pressure plates (Soil Moisture Equipment Co., Santa Barbara, CA).

To study the survival of sporangiospores in the laboratory, 2.5 g of dry soil was placed in sterile glass tubes (15 × 1.8 cm) and maintained at 0, 21, 27, 33, 36, or 39 C under three soil moisture conditions. Each temperature-soil moisture regime was replicated three times. A wet treatment (20 g of water per 100 g of dry soil resulting in approximately -0.3 bar matric potential) was initiated by the addition of the spore suspension. A suspension (0.5 ml) of sporangiospores (3.2×10^4 spores per milliliter) of *M. piriformis* was added to the soil in each tube after keeping them 8 hr at the desired temperature. Soil tubes were sealed with laboratory film (Parafilm M, American Can Company Dixie/ Marathon, Greenwich, CT) and weighed periodically. In a dry treatment, test tubes were left unsealed and the soil was allowed to dry slowly from the original 20 g of water per 100 g of dry soil to 1-1.3 g of water per 100 g of dry soil (resulting in a -1,300 bars matric potential) by the final date of sampling for tubes incubated at 21-39 C. The final soil water content for tubes incubated at 0 C was 6-7 g of water per 100 g of dry soil (-7 to -10 bars matric potential). In an alternately wet and dry treatment, test tubes were left unsealed, weighed

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periodically, and moisture depletion corrected by adding sterile distilled water to 20 g of water per 100 g of dry soil every 15 days in tubes incubated at 0 and 21 C, every 5–6 days in those incubated at 27 and 33 C, and every 2 days in tubes held at 36 and 39 C. Matric potential of dry soil was determined with a thermocouple psychrometer [isopiestic technique (12)].

The soil dilution plate technique was used to determine numbers of surviving sporangiospores in soil. The plates were prepared by spreading evenly 100 μ l of a 1:10,000 soil dilution onto the surface of each of five PDA plates, medium acidified to pH 3.5 \pm 0.1. After 24–26 hr incubation at 18 C, fungal colonies were counted by observing the plates through transmitted light. The petri plates were held at 0–1 C for 4–5 additional days and read again. The numbers of surviving sporangiospores were expressed in percentages of the initial density of sporangiospores. Experiments were repeated twice and the semilogarithmic transformation for survival data as proposed by Dimond and Horsfall (9) was followed. Regression analysis to determine slope values of all curves in natural log of percentage surviving sporangiospores per unit of time (days) and comparisons of effects among temperatures, moistures, and temperature \times moisture interactions were done with analysis of covariance (Statistical Analysis Systems, SAS Institute, Inc., Cary, NC).

Survival in orchard soils. To determine the type and longevity of surviving propagules of *M. piriformis* in orchard soils, 30 ripe peaches (*Prunus persica* (L.) Batsch. 'Elberta') were wound-inoculated with 50 μ l of a 1 \times 10⁵ spore per milliliter suspension of each isolate of *M. piriformis* and incubated at 4 C for a month. After removal of the pits, the peaches, which were completely decayed and covered with sporulating *M. piriformis*, were macerated for 1 min (15,500 rpm) in a Waring commercial blender (Blender 5010S, Waring Products Division, Dynamics Corporation of America, New Hanford, CT). The entire suspension (decayed fruit tissue plus fungal mycelia and sporangiospores) was added to 25 kg of soil (collected from the top 10-cm layer of soil in each peach orchard in Davis and Parlier) and mixed thoroughly by hand with 3 L of distilled water. Approximately 3 kg of this mixture was distributed into each of 12 galvanized-wire (1-cm² opening) baskets (25 \times 17 \times 5.5 cm) lined with a single layer of cheesecloth, stored overnight at 0 C, and buried the following day at depths of 5 and 10 cm below the soil surface. Two baskets containing propagules of the same isolate were placed side by side for each of the three replications at each depth of burial in a complete randomized block design in two rows of peach trees at the Davis and Parlier orchard plots. A flat, rectangular piece of galvanized screen (65 \times 35 cm) of 0.5-cm² was used to cover the baskets before burial. Each soil type was returned to the site of origin. Soil temperature at 5- and 10-cm depths was measured with a two-point thermograph (Model 4020, Weathertronics, Inc., Sacramento, CA).

Contents of the buried baskets were monitored for propagules of *M. piriformis*. Samples of about 20 g were taken before burial and at predetermined intervals using a soil tube sampler (2-cm inner diameter). Before each sampling, the sampler was surface sterilized with a 0.53% sodium hypochlorite solution and dried with a clean paper towel. The soil samples were sealed in plastic bags and transported to the laboratory in an ice chest. Ten grams of each sample was used to determine soil moisture by drying the soil at 105 C for 24 hr. Another 2 g was diluted serially in sterile water and plated on acidified PDA (five replicates of 100 μ l). To determine the kind of propagule unit that had survived, randomly selected colonies, not covered by soil particles, were observed individually under a compound microscope (\times 80) in the dilution plates. The number of germ tubes per colony also was recorded. The mean numbers of propagules per gram of dry soil (averaged from five plates per replication at each sampling time over a year) were analyzed by regression analysis. Data on the change of population with time were examined on arithmetic and semilogarithmic graphs. Simple regressions of the natural log of percentage survivors on sampling time were performed. Correlation coefficients (*r*) were calculated by linear regression methods, and slopes of the lines were statistically compared with a *Z*-value test

(5).

Survival on intact decayed peaches buried in orchard soils. Ripe peaches (cultivar Halloween) were inoculated by placing a drop of spore suspension containing 1 \times 10⁵ spores per milliliter in wounds created with a glass rod (3 mm in diameter). Inoculated peaches were incubated at 4 C for 1 mo until the entire peach was decayed and covered with masses of spores. Six decayed peaches were placed in each basket and covered with soil to a depth of 5 cm below the soil surface. Two microplots of three baskets each of CA and CH isolates were set up at Parlier 1 December 1981 and four microplots of three baskets at the Davis orchard 3 December 1981. Microplots were located beneath peach or nectarine trees in two adjacent tree rows. At 1-mo intervals for 22 mo, three random samples of decayed (mummified) fruit tissue and adjacent soil were collected using sterile forceps from each microplot for each isolate. These samples were placed on acidified PDA slants, incubated at 0–1 C for 20 days, and results recorded.

Survival of fungal mycelia in soil. Vegetative growth of *M. piriformis* was attained by placing six pieces (2.5 cm diameter) of nylon 240- μ m mesh (Nitex 100% polyamide nylon fiber, HC3-240, Tetco Inc., Elmsford, NY) in a petri plate. The nylon pieces were covered with 10 ml of potato-dextrose broth (PDB), autoclaved, seeded with 120 μ l of a 4 \times 10⁵ spore per milliliter suspension of *M. piriformis*, and incubated initially at 15 C for 2 days followed by 0 C for 10 days. The nylon pieces submerged in the fresh PDB at 0 C were extensively colonized by mycelium with no sporulation. The mesh pieces were rinsed in sterile water before being covered with wet (–0.5 bar matric potential) or dry (–1,300 bars matric potential) Hanford fine sandy loam soil in a petri plate. The soil for this study was screened through a 5.6-mm-mesh sieve, air-dried at room temperature for 3–4 days (soil contained 1–2% water at this point), after which 100 g was distributed into deep (10 \times 2.5 cm) petri plates. For wetted soil, 20 ml of sterile distilled water was mixed into the soil with a spatula. Two nylon pieces colonized with *M. piriformis* were transferred onto Whatman No. 1 filter paper to remove excess moisture and buried in each petri plate containing wet soil. Similarly, two nylon pieces were allowed to dry on filter paper for 15–20 min at room temperature, then buried in dry soil. Plates were incubated at 21 C and three replicate plates were harvested after incubation of one to 60 days. Soil attached to the nylon pieces was removed with 10 ml of sterile distilled water, the piece cut aseptically into four equal quarters, plated on acidified PDA, and observed for mycelial growth at the edge of the nylon piece after 24 hr incubation at 18 C. Data were analyzed by using regression analysis with the Statistical Analysis Systems, and comparison of the treatments was done by comparing the slope of the regression lines.

Colonized nylon pieces also were buried in soil at Parlier. Enroute to the field the nylon pieces were submerged in water and kept in an ice chest. Wire baskets lined with a single layer of cheesecloth were half filled with dry (–1,300 bars matric potential) Hanford fine sandy loam soil from the upper 10-cm layer. Then 20 nylon pieces were placed on the soil surface, covered with soil, and buried so that the middle of the basket was positioned approximately 5 cm below the surface of the orchard floor. Five wire baskets were buried in each microplot per fungal isolate. The baskets were buried in four microplots (1 \times 1 m), two wet and two dry. In the wet microplots, the soil was irrigated initially and every week thereafter with 30 L of water. In the dry microplots, the soil was not wetted throughout the experiment. Initial sampling began a day after burial 16 August 1983 and at weekly intervals thereafter for 4 wk, until the supply of baskets was depleted. On return to the laboratory, the nylon pieces were plated onto acidified PDA plates, using the method previously described, and *M. piriformis* growth from their edges was recorded. Soil temperature and moisture at the 5-cm depth were measured as previously described.

RESULTS

Survival of sporangiospores in soil at various temperatures and moistures. Survival of sporangiospores was not dependent on soil type (*P* > 0.10) and the two isolates showed similar trends in

viability loss. Therefore, results for sporangiospore survival under controlled conditions reflect those obtained with the Reiff loam soil and the CA isolate.

In general, survival of sporangiospores of *M. piriformis* decreased with incubation time and decreased most slowly at 0 and 21 C (smallest negative slope values, Fig. 1A and B) and fastest at 36 and 39 C (largest negative slope values, Fig. 1E and F). At 27 and 33 C, the decline of surviving sporangiospores had an intermediate rate between 0 and 21 C and 36 and 39 C (most of the slopes had intermediate values, Fig. 1C and D). Comparison of the slopes of the lines indicates that there were no significant differences between survival rates in dry, wet, and wet-dry soil at 0, 36, and 39 C (Fig. 1A, E, and F), or between wet or wet-dry treatments at 21 and 33 C (Fig. 1B and D). At 27 C, however, all three slopes were significantly ($P=0.05$) different with the slope of the wet soil having the smallest value (Fig. 1C). The moisture \times temperature interaction was significant ($P=0.05$) for 21, 27, and 33 C, but not for 0, 36, and 39 C. All r^2 values were significant at $P<0.05$ except those of the three regression lines at 0 C and the lines of dry and wet-dry treatments at 21 and 27 C (Fig. 1A-C). Although both fungal isolates showed similar trends in viability loss, sporangiospores of the CA isolate survived at significantly ($P=0.01$) higher inoculum density than the CH isolate in temperatures from 0 to 33 C. No statistical differences were found between the isolates at 36 and 39 C.

Survival in orchard soils. Arithmetic plots of the percentage of surviving propagules with sampling time were curvilinear. At both

test orchards the number of viable propagules of *M. piriformis*, buried at either 5- or 10-cm soil depths, declined rapidly during the first 4 wk and gradually thereafter. When percentages of propagules surviving were transformed to natural logarithms and plotted against sampling time expressed in days, a straight line relationship was obtained (Fig. 2A and B). The negative slopes of the lines represent the rate of decline of propagules expressed in \log_e of the percentage of surviving propagules per day. A comparison of the slopes of the lines of survivors of the same isolate for the two depths or of the two isolates for the same depth of burial at Davis field plot indicated that they did not differ significantly from each other ($P>0.1$), suggesting that neither the depth nor the isolate had a significant effect on the levels of viable propagules after a year. At Parlier, propagules of the CA isolate at 10-cm depth declined more slowly ($P<0.01$) than propagules of the CA isolate at 5 cm or propagules of the CH isolate at either depth (determined by comparison of the slopes of regression lines). In addition, the slopes of the regression lines for the data obtained at Parlier for any one treatment were greater negative ($P<0.05$) than those of the same treatments at Davis, indicating that the decline was faster at Parlier.

The weekly mean and maximum soil temperatures recorded at 5- and 10-cm depths at Parlier were generally higher (3-8 C) than those recorded at Davis. Temperatures at 5- and 10-cm depths did not differ at Davis, whereas greater weekly mean and maximum temperatures were recorded at 5- than at 10-cm depth in soil at Parlier. No differences were evident in soil moisture ranges at 5-

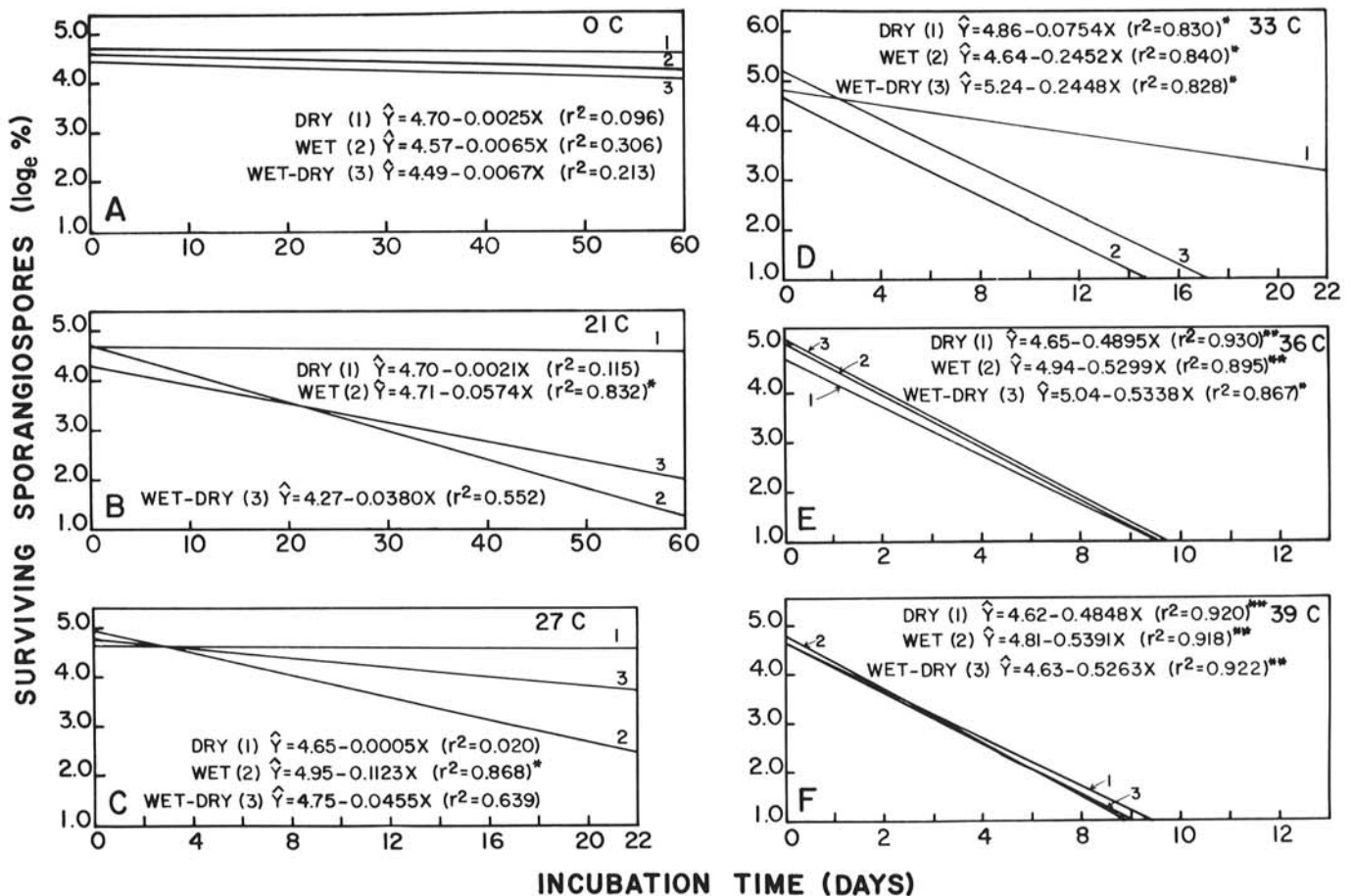


Fig. 1A-F. Regression lines representing the survival of sporangiospores of *Mucor piriformis* [California isolate (CA)] incubated for various lengths of time at constant temperatures and various moistures in a Reiff loam soil. Temperature was maintained at A, 0 C; B, 21 C; C, 27 C; D, 33 C; E, 36 C; and F, 39 C. Soil initially at 20 g of water per 100 g of air-dried soil (-0.3 bar matric potential) was allowed to dry to 6-7 g of water per 100 g of dry soil (-7 to -9 bars matric potential) in tubes incubated at 0 C for 60 days and to 1-1.3 g of water per 100 g of dry soil (-1,300 bars matric potential) in tubes incubated at 21 C for 60 days, at 27 and 33 C for 22 days, and at 36 and 39 C for 10 days [dry, lines (1)]; or soil moisture was maintained continuously at 20 g of water per 100 g of dry soil [wet, lines (2)]; and soil initially at 20 g of water per 100 g of dry soil was watered to restore the initial water content every 15 days in tubes incubated at 0 and 21 C, every 6 days in tubes incubated at 27 and 33 C, and every 2 days in tubes incubated at 36 and 39 C [wet-dry, lines (3)]. \hat{Y} in the equations of the regression lines refers to the \log_e of the percentage of surviving propagules and 'X' to incubation time in days. (* = significance at $P=0.05$, ** = significance at $P=0.01$).

and 10-cm depths in the Davis plot, but the soil moisture at 10-cm depth was 5–7% greater than at 5 cm at Parlier during the summer of 1983.

Survival on intact decayed peaches buried in orchard soils. Survival of isolate CA and CH was 100 and 50%, respectively, after 20 mo on intact fruit buried in the soil at Davis (Table 1). At Parlier, propagules of the CA isolate were recovered in 17% of the samples after 19 mo, but not after 20 mo. In contrast, propagules of the CH isolate remained viable for only 9 mo, i.e., in 83% of the samples (Table 1). Examination of soil dilution plates revealed that all colonies emerged from germinating sporangiospores producing 1–3 germ tubes.

Survival of fungal mycelia in soil. Viable mycelia of isolates CA and CH were recovered from the Hanford fine sandy loam in the laboratory at 21 C after 30–40 and 30 days, respectively, in wet (–0.5 bar matric potential) or dry (–1,300 bars matric potential) soil. Arithmetic plots of the percentage of surviving mycelia with incubation time in soil were curvilinear (assuming that survival of mycelia is equal to the success recovery of viable mycelia). When the percentages of surviving mycelia were transformed to natural logarithms and plotted against incubation time expressed in days, a straight line relationship was obtained (Fig. 3). All correlation coefficients were significantly positive ($P = 0.01$) in the transformation. The negative slopes of the lines represent the rate of decline of surviving mycelia expressed in \log_e of the percentage of survivors per day. The decline of surviving mycelia was slower ($P < 0.01$) in dry than in wet soil. Under the same soil moisture treatment, the survival of mycelia of isolate CA and CH did not differ significantly ($P > 0.1$).

In general, after 3 days of incubation in soil, mycelia appeared healthy, but after 5–10 days, mycelial protoplasts appeared fragmented (Fig. 4A), occasionally granular and retracted (Fig.

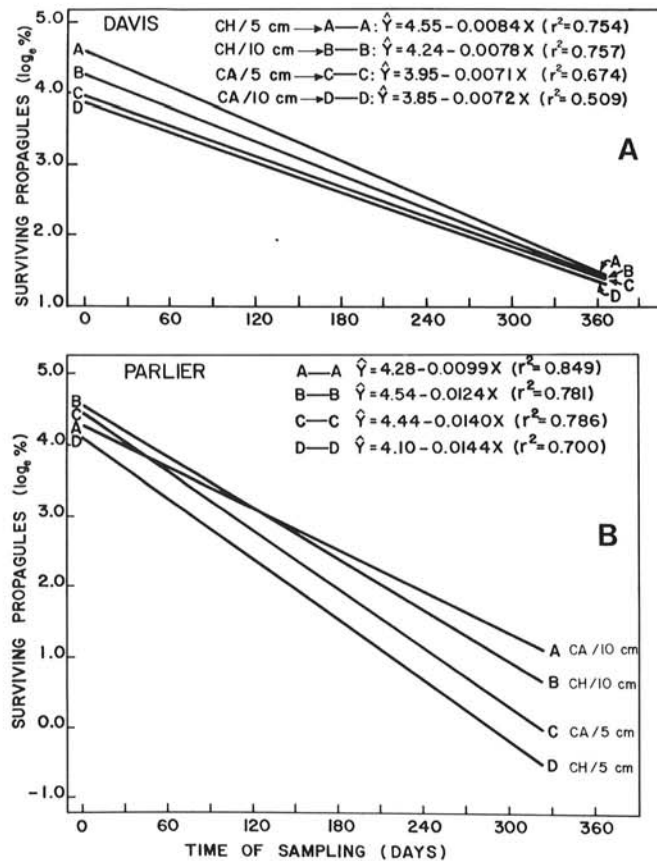


Fig. 2. Regression lines representing the decline of surviving propagules of *Mucor piriformis* [California isolate (CA), Chile isolate (CH)] buried at 5- and 10-cm depths in two peach orchards A, Davis and B, Parlier. \hat{Y} in the equations of the lines refers to the \log_e of the percentage of surviving propagules and 'X' to the time of sampling in days; all r^2 are significant at $P = 0.01$.

4B–E), and with unusual septation (Fig. 4F). A breakdown of the mycelial wall was evident by the fourth day of soil incubation and most of the hyphae were lysed by the 15th day.

In field experiments at Parlier, viable mycelia of both isolates were recovered after 7 days in wet (approximately –1.0 bar matric potential) soil. In dry (–1,300 bars matric potential) soil, nylon pieces containing CA isolate exhibited vegetative growth after 21 days. Viable mycelia of CH isolate were not recovered after 7 days.

DISCUSSION

As a rule, cold and dry conditions contribute to long-term survival of fungi and other microbes (16). Our results with sporangiospores of *M. piriformis* did not differ in this regard. Propagules survived best in dry conditions, especially when temperatures were 27 C or less. Presumably, antagonistic microflora were more active in wet soil. However, irrespective of soil moisture regimes, soil temperatures of 33 C and above caused a

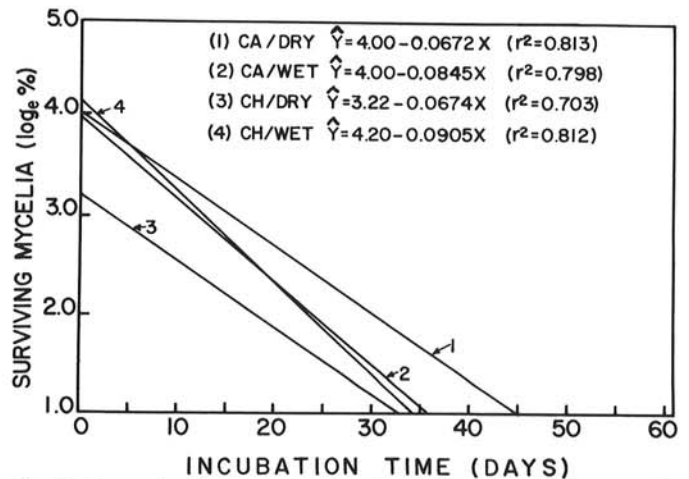


Fig. 3. Regression lines representing the decline of surviving mycelia (determined by percentage success recovery) of *Mucor piriformis* [California isolate (CA), Chile isolate (CH)] in a Hanford fine sandy loam soil in the laboratory under wet (–0.5 bar matric potential) and dry (–1,300 bars matric potential) conditions at 21 C for 60 days. \hat{Y} in the equations of the lines refers to the \log_e of the percentage of surviving mycelia and 'X' to the incubation time in days; all r^2 are significant at $P = 0.01$.

TABLE 1. Success recovery of propagules of *Mucor piriformis* [California isolate (CA), Chile isolate (CH)] from entirely decayed peaches buried intact in baskets 5 cm deep in soil at Davis and Parlier, CA

Sampling time (date)	Time from date of burial (mo)	Location ^a , isolate, and % success recovery of viable propagules ^b			
		Davis		Parlier	
		CA	CH	CA	CH
Dec. 1981	1	100	100	100	100
Jan.–May 1982	2–6	100	100	100	100
Jun. 1982	7	100	83	100	100
Jul. 1982	8	100	100	100	100
Aug. 1982	9	83	100	100	83
Sep. 1982–Feb. 1983	10–15	100	100	100	0
Mar. 1983 ^c	16	100	0
Apr. 1983	17	100	100	100	0
May 1983	18	100	67	100	0
Jun. 1983	19	100	33	17	0
Jul. 1983	20	100	50	0	0
Aug. 1983 ^d	21
Sep. 1983	22	0	0	0	0

^a Two microplots of three baskets each of isolates CA and CH were set up at Parlier on 1 December 1981 and four microplots of three baskets at Davis orchards on 3 December 1981.

^b Average of three replicate isolations from each microplot.

^c No sampling was done for that month at Davis because soil was saturated by extensive precipitation in March 1983.

^d No sampling.

rapid decline in sporangiospore viability (Fig. 1D-F). Nickerson et al (24) reported that wet sporangiospores of *Rhizopus stolonifer* (Ehr.:Fr.) Lind did not maintain viability compared with dry spores during storage at room temperature. Their results parallel our findings with *M. piriformis*.

Studying other fungi, Stanghellini and Hancock (27) showed that fluctuating or constant soil moisture regimes have little effect on the survival of sporangia of *Pythium ultimum* Trow. Our results showed that continuously wet and alternately wet-dry soil conditions did not significantly alter sporangiospore viability. Reports on the survival of other fungi (14,28) show that alternately wet-dry soil is less favorable for survival of fungal spores than soil consistently wet or dry.

The orchard soil in Davis was 3-8 C cooler than that in Parlier. At Davis, where weekly mean temperature was lower than 27 C during the season, the initial decline of viable propagules was 20-40% and viability was retained for 52 wk. In contrast, temperatures at Parlier reached 27 C and higher and the initial decline was 60% (after 4 wk in soil). Propagules were not recovered except for those of isolate CA at 10-cm depth after 41 wk, when weekly mean and maximum temperatures of soil were again about 27 C or greater. The survival of propagules of isolate CA only at a 10-cm depth after 41 wk at Parlier may be explained by the fact that this isolate is less sensitive to temperature (22). No great differences were recorded in moisture ranges in soil at 5- and 10-cm depths, either at Davis or Parlier, except during the summer of 1983 at Parlier. Soil moisture at Davis was generally higher than that at Parlier because of the higher water-holding capacity of the Reiff loam soil. Longer survival of *M. piriformis* at Davis can be attributed to temperature differences because, if moisture was the factor, one would expect more surviving propagules at Parlier with the drier soil.

Mathematical equations and transformations have been proposed to describe survival of plant pathogens (1,9). In this study, the semilogarithmic transformation as proposed by Dimond and Horsfall (9) applied well to most of the results of surviving sporangiospores in the laboratory (Fig. 1) and to all the results in the field, regardless of the isolate, depth of burial in soil, or location (Fig. 2A and B). The smaller negative slopes of the regression lines of propagules buried in soil at Davis compared with those buried at Parlier indicate the slower decline and eventually the longer survival of propagules at Davis. At present, data are not sufficient to predict propagule survival based on soil temperature, moisture, and/or time. The mathematical models and regression equations from our field trials were analytical rather than predictive, but the exponential patterns indicate initial rapid decline of surviving propagules that reach a constant residual level.

Propagules of CH isolate survived 9-9.5 mo in a mixture of inoculum from macerated fruit with soil and on intact peaches buried 5 cm deep in soil at Parlier. In contrast, propagules of CA isolate survived longer (up to 19 mo) on intact peaches than on a mixture of inoculum of macerated fruit and soil when both were buried at a 5-cm depth in the soil. No additional sporulation was observed microscopically on samples of the mixture of macerated fruit and soils. The development of additional sporulation on intact peaches can explain the longer survival of *M. piriformis* on intact fruit. This indicates that in peach orchards, such as the one at Parlier, *M. piriformis* in decayed fruit on the orchard floor could be buried in the soil during cultivation and survive until the following season. However, if decayed fruit on the orchard floor was crushed in some manner, mixed with the soil by discing, and then buried, the chances for survival of propagules (even of CA isolate) until the following season would be minimal.

These results indicated that sporangiospores rather than mycelia are the long-term survival structures of *M. piriformis*. Sporangiospores were capable of surviving in soil up to at least 1 yr and, therefore, their survival would be sufficient to carry the inoculum from 1 yr to the next in peach orchards. The ephemeral nature of fungal mycelia is well documented (19). The short survival of mycelia of *M. piriformis* in soil indicates that mycelium is not important in long-term survival, but may be important in

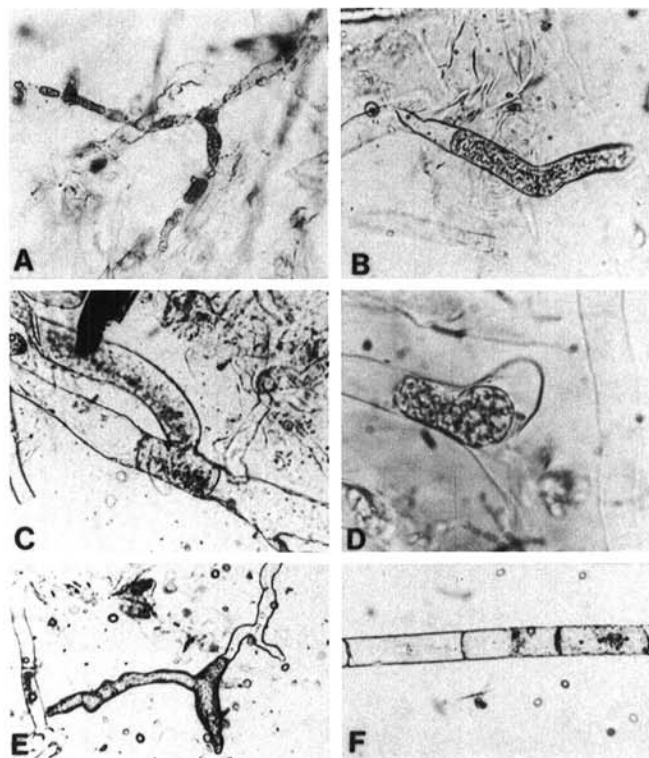


Fig. 4A-F. Microscopic characteristics of mycelium of *Mucor piriformis* buried in a Hanford fine sandy loam soil of -0.5 bar matric potential for 5-10 days at 21 C in darkness. A, Fragmentation of protoplast in mycelium of CA isolate after 10 days ($\times 125$); B and C, Retraction of protoplast in mycelium of CA after 7 days ($\times 175$); D and E, Retraction of protoplast in mycelium of CH isolate after 7 days (D, $\times 200$ and E, $\times 65$); F, Unusual septation in hypha of CA isolate after 5 days ($\times 175$).

building up the level of sporangiospores after successful colonization of a host (fruit on the ground). Extensive lysis of mycelia was obvious after 15 days incubation in soil and the percentage of recovery of viable mycelia dropped to about 15% or lower thereafter. Survival of mycelia up to 40 days could be attributed to retracted protoplasts. Although the exact role of retracted protoplasts was not determined in this study, it has been reported that similar retraction of protoplasts was observed in germ tubes of sporangia from *P. ultimum* in artificially infested soil, and it was suggested that the retracted protoplasts acted as a survival mechanism in countering lysis in soil (27). Because *M. piriformis* does not produce any chlamydospores (11), it is not surprising that sporangiospores, which are produced in great numbers, serve the role of the long-term survival propagules of *M. piriformis* in the orchard soil. Our finding that sporangiospores of *M. piriformis* are the long-term survival structures in soil should help in developing efficient control methods. Although *M. piriformis* is not expected to be a serious postharvest fruit pathogen in California, because of the unfavorable hot climatic conditions in frequently irrigated orchards, it is very important in cold areas such as Oregon and Washington (3).

LITERATURE CITED

1. Baker, R. 1971. Simulators in epidemiology—Can a simulator of root disease be built? Advanced Study Institute: Epidemiology of Plant Diseases, Wageningen, Netherlands 1:1-2.
2. Bertrand, P., and Saulie-Carter, J. 1979. Postharvest decay control of apples and pears after immersion dumping. Ore. State. Univ. Exp. Stn. Spec. Rep. 545. 9 pp.
3. Bertrand, P., and Saulie-Carter, J. 1980. Mucor rot of pears and apples. Ore. State Univ. Exp. Stn. Spec. Rep. 568. 21 pp.
4. Bisby, G. R., James, N., and Timonin, M. 1933. Fungi isolated from Manitoba soil by the plate method. Can. J. Res. 8:253-275.
5. Caulcott, E. 1973. Significance Tests. Routledge and Kegan Paul, London and Boston. 145 pp.
6. Christenberry, G. A. 1940. A taxonomic study of the Mucorales in the

- southeastern United States. *Elisha Mitchell Sci. Soc.* 56:333-366.
7. Colhoun, J. 1938. Fungi causing rots of apple fruit in storage in northern Ireland. *Ann. Appl. Biol.* 25:88-99.
 8. Dennis, C., and Blijham, J. M. 1980. Effect of temperature on viability of sporangiospores of *Rhizopus* and *Mucor* species. *Trans. Br. Mycol. Soc.* 74:89-94.
 9. Dimond, A. E., and Horsfall, J. G. 1965. The theory of inoculum. Pages 404-415 in: *Ecology of Soil-borne Plant Pathogens*. K. F. Baker and W. C. Snyder, eds. University of California Press, Berkeley. 571 pp.
 10. Domsch, K. H., and Gams, W. 1972. *Fungi in Agricultural Soils*. Longman Group Ltd., London. 290 pp.
 11. Domsch, K. H., Gams, W., and Anderson, T-H. 1980. *Compendium of Soil Fungi*. Vol. 1. Academic Press, New York. 859 pp.
 12. Duniway, J. M. 1975. Limiting influence of low water potential on the formation of sporangia by *Phytophthora drechsleri* in soil. *Phytopathology* 65:1089-1093.
 13. Edney, K. L. 1964. Postharvest rotting of strawberries. *Plant Pathol.* 13:87-89.
 14. Ellerbrock, L. A., and Lorbeer, J. W. 1977. Survival of sclerotia and conidia of *Botrytis squamosa*. *Phytopathology* 67:219-225.
 15. English, H., and Heald, F. D. 1936. The fungi causing decay of pears in Washington. (Abstr.) *Phytopathology* 26:92.
 16. Garrett, S. D. 1956. *Biology of Root-infecting Fungi*. Cambridge University Press, Cambridge, England. 293 pp.
 17. Heald, F. D., and Ruehle, G. D. 1931. The rots of Washington apples in cold storage. *Wash. State College Exp. Stn. Bull.* 253. 47 pp.
 18. Jensen, H. L. 1931. The fungus flora of the soil. *Soil Sci.* 31:124-158.
 19. Lloyd, A. B., and Lockwood, J. L. 1966. Lysis of fungal hyphae in soil and its possible relation to autolysis. *Phytopathology* 56:595-602.
 20. Lopatecki, L. E., and Peters, W. 1972. A rot of pears in cold storage caused by *Mucor piriformis*. *Can. J. Plant Sci.* 52:875-879.
 21. Michailides, T. J. 1980. Studies on postharvest decay of stone fruit caused by *Mucor* species. M.S. thesis. University of California, Davis. 63 pp.
 22. Michailides, T. J., and Ogawa, J. M. 1982. A comparative study of growth characteristics and pathogenicity of *Mucor piriformis* isolates causing decay of peaches and nectarines. (Abstr.) *Phytopathology* 72:1008.
 23. Michailides, T. J., and Ogawa, J. M. 1983. Survival of *Mucor piriformis* in soil. (Abstr.) *Phytopathology* 73:803.
 24. Nickerson, K. W., Frees, S. W., and van Etten, J. L. 1981. *Rhizopus stolonifer* sporangiospores: A wet harvested spore is not a native spore. *Exper. Mycol.* 5:189-192.
 25. Smith, W. L., Jr., Moline, H. E., and Johnson, K. S. 1979. Studies with *Mucor* species causing postharvest decay of fresh produce. *Phytopathology* 69:865-869.
 26. Spotts, R. A. 1985. Environmental factors affecting conidial survival of five pear decay fungi. *Plant Dis.* 69:391-392.
 27. Stanghellini, M. E., and Hancock, J. G. 1971. The sporangium of *Pythium ultimum* as a survival structure in soil. *Phytopathology* 61:157-164.
 28. Vizvary, M. A., and Warren, H. L. 1982. Survival of *Colletotrichum graminicola* in soil. *Phytopathology* 72:522-525.