Effects of High Temperatures on the Survival and Pathogenicity of Propagules of *Mucor piriformis*

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ABSTRACT

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Survival of mycelia and sporangiospores of *Mucor piriformis* (California isolate [CA] and Chile isolate [CH]) were compared at temperatures of 35–60 C. The thermal death points of the mycelia and sporangiospores were 46 and 55 C, respectively, for isolate CA and 43 and 52 C, respectively, for isolate CH. After a 2-day incubation at 27 C, both isolates exhibited yeastlike growth on agar medium. Sporangiospore germination was erratic at 27 C and, when subsequently incubated at 21 C, germ tubes were abnormally swollen and produced no viable colonies. Reduction in viability was greater in wet than in dry sporangiospores. Preincubation of sporangiospores in dry (−1,300 bars matric potential) or wet (−0.3 bar matric potential) soil at 27 or 33 C for 15 days followed by incubation at 21 C for 45 days resulted in significantly lower viability in both isolates than in sporangiospores incubated continuously at 21 C for 60 days at both water potentials. Preincubation of sporangiospores at 33 C for 15 days resulted in a faster decline in survival than preincubation at 27 C for 15 days followed by 21 C for 45 more days. Pear fruits wound-inoculated with *M. piriformis* and dipped in 47 C water for 30 min had 1–5% infected wounds, whereas fruits inoculated in the same way and dipped for 30 min in water at 21 C had 90% of the wounds infected. Results from this study suggest that hot water treatment of fruit may reduce inoculum levels and postharvest infection.

Additional keywords: Mucor decay, postharvest pathogen.

Fungi are sensitive to temperature extremes below and above their optima, which either lower their viability or are lethal (23). In recent years, interest in the use of thermotherapy to control postharvest decay has increased. The use of heat alone or in conjunction with radiation resulted in significantly lower decay of fruit by *Rhizopus* or *Monilinia*, and the heat treatment predisposed the spores to inactivation by radiation (26).

Mucor piriformis Fischer is a soilborne fungus and a common postharvest pathogen of pears and apples (5,6,7,16). Occasionally, however, the fungus can cause losses of peaches, nectarines (17,25), and strawberries (10). The fungus can cause postharvest fruit decay at 0–20 C (17,25), and its maximum temperature limit for mycelial growth is 27 C (17).

Smith et al (25) reported the apparent death of sporangiospores and mycelia of *M. piriformis* on potato-dextrose agar (PDA) and observed yeastlike growth from mycelial plugs of some of the isolates when incubated at 27 C. Michailides and Ogawa (19) determined that sporangiospores of *M. piriformis* did not survive well at soil temperatures of 27 C or above. In a peach orchard where the mean weekly soil temperatures were 27 C or higher during the summer months, only 40% of the sporangiospores survived after 4 wk and none after 1 yr. However, in another orchard where soil temperatures were below 27 C during the same period, 2–6% of the initial population of sporangiospores survived after 1 yr (19). The fast decline of sporangiospores was more pronounced in wet soils than in dry soils, although soil temperature was more important than moisture (19). The purpose of the present study was to determine the effects of high temperature on the germination, growth, and survival of propagules of *M. piriformis*. In addition, a hot water dip was used to control decay on pear fruit.

MATERIALS AND METHODS

Isolates. Two isolates of *M. piriformis* were used: isolate CA (ATCC 52555) was obtained from an infected peach in California, and isolate CH (ATCC 52554) was from a nectarine shipped from Chile to California (19).

Effect of temperatures above 27 C on survival of sporangiospores. These experiments were conducted in a water bath (Magni Whirl, Constant Temperature Bath with Blue M Microtrol, Blue M Electric Co., Blue Island, IL). Sporangiospores of *M. piriformis* were produced on acidified (pH = 3.5 ± 0.1) potato-dextrose agar (APDA) cultures incubated at 21 C for 5 days, and 0.5 ml of a spore suspension (2×10^6/ml) was added to 25-ml flasks containing 2 ml of distilled water and incubated for 10 min at temperatures ranging

from 35 to 60 C. The flasks were preincubated in the water bath for 1 hr at each temperature before the spore suspension was added. Survival of heat-treated sporangiospores was determined from the percentage germination on five APDA plates after 15 hr at 21 C. Plates with very low sporangiospore germination were also observed after 33–48 hr and colonies per plate were recorded.

Effect of temperatures above 27 C on survival of mycelia. To determine the survival of mycelia at high temperatures, mycelial colonies of *M. piriformis* (0.5–1 cm in diameter) were initiated by incubating APDA plates at 18 C for 24 hr and subsequently at 0 C for 4–5 additional days to permit further hyphal growth without sporulation. Mycelial colonies were removed from the medium by flooding the APDA plates with sterile distilled water and washed in a four-layer cheesecloth with sterile distilled water to reduce nutrients from the medium contaminating the mycelium. The mycelial colonies were resuspended in sterile distilled water. Two to three intact colonies were rolled on the tip of a transfer needle, which was dipped for 10 min in 25-ml flasks containing 10 ml of distilled water preincubated in a water bath for 1 hr at temperatures of 30–50 C. Survival was determined by plating the treated mycelia on five replicate APDA plates. New growth developing from plated mycelia after 24 hr of incubation at 21 C indicated survival, whereas no growth indicated killed mycelia.

Behavior and survival of sporangiospores at prolonged exposure to 27 C. *Wet spores.* For these experiments, synthetic Mucor agar (SMA) was used (13). Descriptions for both isolates of *M. piriformis* grown on this medium under optimum temperature (21 C) were given previously (17). A sample (100 μl) of a suspension containing $1.2–1.3 \times 10^4$ spores per milliliter from a 4-day-old culture of *M. piriformis* was spread on SMA in each plastic Petri plate. Plates held from 0 to 8 days at 27 C were transferred to 21 C to stimulate spore germination. Germination characteristics of the spores were noted at the time of transfer and after 24 hr at 21 C. One hundred spores were counted on each of four replicate plates, and the length of the germ tubes for 10 randomly selected sporangiospores of both isolates was determined after incubation at 27 or 21 C for 18 hr.

Dry spores. Two to three sporangia from each isolate were removed with a glass rod from a 4-day-old culture grown on SMA, placed in a 1-cm-wide × 0.5-cm-high glass vial, and air dried for 15 min at room temperature and 40–45% RH. After 2, 4, 6, 8, and 10 days of incubation at 21 or 27 C, two vials were removed and 0.3 ml of distilled water was added to each. Samples (100 μl) of the resulting spore suspension were spread on SMA plates and incubated at 21 C. Spore germination was determined after 24 hr of incubation. Results for this and the above experiments are the average of two repeated experiments. Regression analysis was conducted to describe log of percent of abnormal and normal germination of sporangiospores in relation to days of their preincubation at 27 C.

Predisposition and survival of sporangiospores in soil. The soil used for these experiments was a Hanford fine sandy loam (14% clay, 25% silt, and 61% sand) from the University of California Kearney Agricultural Center at Parlier. The soil was 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 for the survival experiments without autoclaving. Percent water content at field capacity determined with ceramic pressure plates (Soil Moisture Equipment Co., Santa Barbara, CA) was 24% for this soil.

To study the effects of predisposition on survival of sporangiospores, 2.5 g of dry soil was placed in sterile glass tubes (15 × 1.8 cm) and equilibrated at temperatures of 21, 27, or 33 C for 8 hr before spores were introduced. A wet and a dry moisture condition were studied. The wet treatment, consisting of 20 g of water per 100 g of dry soil (−0.3 bar matric potential), was initiated by adding a suspension (0.5 ml per test tube) of sporangiospores (3.2×10^4 spores per milliliter) of *M. piriformis*. Soil tubes were sealed with Parafilm M (American Can Company Dixie/Marathon, Greenwich, CT) and weighed periodically. In the 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 (−1,300 bars matric potential) by the final date of sampling. Matric potential of dry soil was determined with a thermocouple psychrometer (isopiestic technique [9]). Tubes incubated at 27 and 33 C for 15 days were then transferred at 21 C for 45 more days; control test tubes were incubated at 21 C for 60 days.

The soil dilution plate technique was used to determine numbers of surviving sporangiospores in soil. The plates were prepared by evenly spreading 100 μl of a 1:10,000 soil dilution from each treatment onto the surface of each of five APDA plates. After 24 to 26 hr of incubation at 18 C, fungal colonies were counted. The Petri plates were held at 0–1 C for 4–5 additional days and colonies were recounted. All experiments were repeated once; results are the average of the two tests. The numbers of surviving sporangiospores were expressed in percentages of the initial density of sporangiospores and transformed to logs (8). Regression analysis was conducted to describe log of percent surviving sporangiospores in relation to days. Effects of the two temperatures, soil moistures, and temperature × moisture interactions were determined with analysis of covariance (Statistical Analysis Systems, SAS Institute, Inc., Cary, NC).

To estimate the initial level of viable sporangiospores in soil, three test tubes per treatment (a total of 15 plates each) were assayed immediately after the addition of a spore suspension.

Effect of hot water treatment on decay of fruits caused by *M. piriformis*. Pears (cultivar Anjou) were surface sterilized in 400 μg of sodium hypochlorite (NaOCl) per liter of water for 3 min and air dried on a laboratory bench. Each fruit was wounded at four areas with a glass rod (2 mm in diameter), inoculated with 20 μl of a suspension containing 1×10^5 spores per milliliter, and incubated at room temperature (22 ± 1 C) for 3 hr. Inoculated fruits were then dipped for 30 min in a water bath in which the temperature was adjusted and maintained at 47 C. Three replicates of six fruits each were used per treatment, and the experiment was repeated twice. After treatment, the fruits were placed in plastic containers and incubated at 20 C and 90% RH. Percentage of infected fruits and the number of infected wounds were recorded after 5 days of incubation. Inoculated fruits dipped in sterile distilled water at 21 C for 30 min served as controls. Treatments were compared with a t pairwise test at $P = 0.05$.

RESULTS

Effect of temperatures above 27 C on survival of sporangiospores and mycelia. Sporangiospores survived at 35–45 C with a 5–10% reduction in viability after 10 min (Fig. 1A); at 50 C, 60% of the sporangiospores remained viable. Temperatures above 50 C reduced viability of the majority of sporangiospores, and at 52 and 55 C none of the sporangiospores of CH and CA isolates, respectively, survived (Fig. 1A). When mycelia were exposed to temperatures above 27 C and below 40 C for 10 min, 90–100% remained viable; however, only 40% were viable after exposure to 41 C (Fig. 1B). At 43 and 46 C, none of the mycelia of CH and CA isolates, respectively, survived (Fig. 1B).

Behavior and survival of sporangiospores at prolonged exposure to 27 C. *Wet spores.* Incubation of sporangiospores of both isolates at 27 C for 1–8 days impaired germination and affected growth of germinated spores when they were later transferred to 21 C (Fig. 2A and B). The abnormally germinating sporangiospores failed to develop into normal colonies. The equations of the regression lines for the CA and CH isolates were $\hat{y} = 1.952–0.0815x$ ($r = -0.960$ [$P < 0.01$]) and $\hat{y} = 1.515–0.1945x$ ($r = -0.875$ [$P < 0.01$]), respectively, where x = log of the percentage of sporangiospore germination. Impairment of spore germination was greater for isolate CH (significantly [$P < 0.05$] greater negative slope) than for isolate CA (Fig. 2A and B). The few sporangiospores that did germinate at 27 C exhibited swollen, multibranched germ tubes (abnormal germination, Fig. 3A and B) or yeastlike growth (Fig. 3C–E). Division by fission was also evident (Fig. 3F). In addition, the average germ tube lengths (±SE) of sporangiospores of isolates CA and CH were 115.2 ± 10.3 μm

and 57.6 ± 6.9 μm, respectively, after 18 hr of incubation at 27 C. In contrast, germ tube lengths of sporangiospores incubated at 21 C for 18 hr were 750.1 ± 137.2 μm and 754.6 ± 67.1 μm for isolates CA and CH, respectively.

Dry spores. The effect of exposure to 27 C on dry sporangiospores held in vials for 1–10 days was less detrimental (Fig. 2C). Sporangiospores of isolate CA had only 10% reduction in germination after 2 days of incubation at 27 C. Sporangiospores of isolate CH had 40–50% germination after 1–8 days and about 30% after 10 days at 27 C. The equations of the regression lines for CA and CH isolates were $\hat{y} = 1.980 - 0.0035x$ ($r = -0.682$ [$P > 0.05$]) and $\hat{y} = 1.827 - 0.0333x$ ($r = -0.760$ [$P < 0.05$]), respectively. Germinated sporangiospores had normal or swollen and multibranched (abnormal) germ tubes. The equations of the regression lines for normally germinated sporangiospores of CA and CH isolates were $\hat{y} = 1.305 - 0.0986x$ ($r = -0.973$ [$P < 0.01$]) and $\hat{y} = 0.323 - 0.2580x$ ($r = -0.849$ [$P < 0.05$]), respectively. Percentage of normally germinated sporangiospores decreased with increased incubation time and decreased more for the CH (significantly [$P < 0.05$] greater negative slope) than for the CA isolate (Fig. 2D).

Heat predisposition and survival of sporangiospores in soil. After 15 days at 33 C, 4 and 0% of the sporangiospores of isolates CA and CH, respectively, remained viable in wet soil, and 50 and 14%, respectively, remained viable in dry soil (Fig. 4A and B). After 15 days at 27 C, 45 and 24% of the sporangiospores of isolates CA and CH, respectively, remained viable in wet soil, and 60 and 42%, respectively, remained viable in dry soil (Fig. 4C and D). After the temperature was lowered to 21 C on day 15, the viability of sporangiospores of both isolates continued to decline (Fig. 4C and D). Sporangiospores of both isolates survived better in soil held at 21 than at 27 C for the first 15 days; at 21 C, there was only a 5–15% reduction in viability in 15 days (Fig. 4E and F). In soil held continuously at 21 C, sporangiospores of both isolates retained their viability for at least 60 days in dry soil, but in wet soil only 25–30% of the sporangiospores of both isolates were still viable after 60 days.

In general, survival of sporangiospores of *M. piriformis* decreased faster in wet soil after a 15-day predisposition at 33 or 27 C (largest negative slope values are Fig. 5A–D) than in dry soil and slower with continuous incubation at 21 C (smallest negative slope values are Fig. 5E and F). Comparison of the slopes of the lines indicated that significant differences existed between survival rates in dry and wet soil at 21 C continuous incubation and after a 15-day predisposition at 27 or at 33 C. The moisture × temperature interaction was significant ($P < 0.05$). All correlation coefficients (r) were significant at $P < 0.05$, except that of the line of wet treatment predisposed at 33 C (Fig. 5A) and those of the regression lines for the dry treatments at 21 C (Fig. 5E and F). Although both fungal isolates showed similar trends in viability reduction, sporangiospores of CA isolate survived at levels significantly ($P < 0.01$) greater than those of CH isolate.

Effect of hot water treatment on pear fruit decay caused by *M. piriformis*. Hot-water treatment resulted in fewer decayed pears (4%) than for the controls (100% were infected) after 5 days of incubation at 20 C (Fig. 6). Approximately 90% of the wounds were infected in control pears, whereas only 1.4% of those dipped in hot water (47 C) for 30 min showed infection (Fig. 6). Hot-water treated pears did not show symptoms of epidermal or fruit flesh damage.

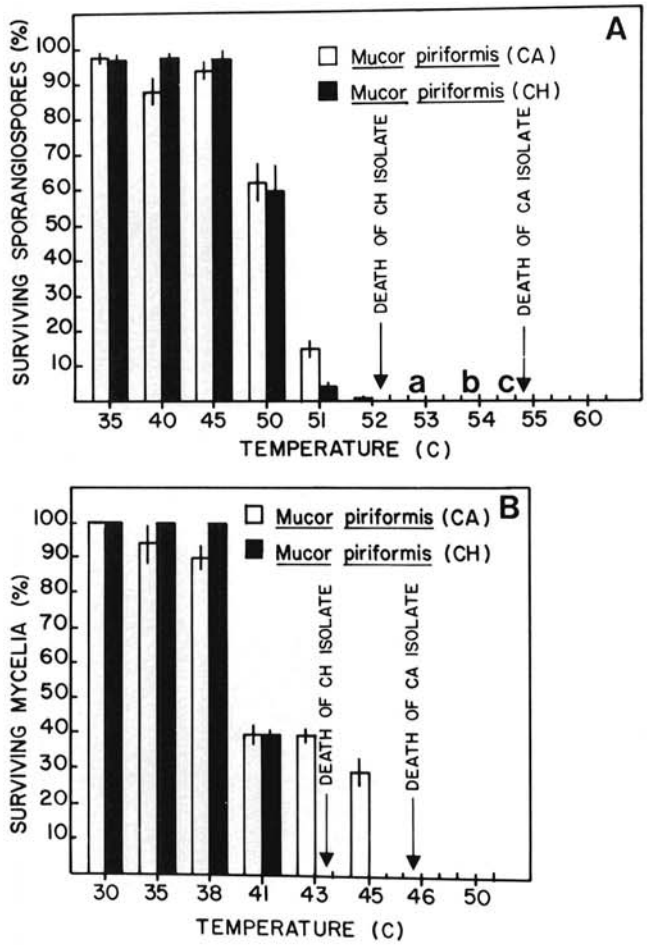

Fig. 1. Survival of *Mucor piriformis* (California isolate [CA], Chile isolate [CH]) in glass-distilled water incubated at temperatures above 27 C for 10 min. **A,** Survival of sporangiospores (only the CA isolate survived above 52 C, producing 10–30 [a], 3–9 [b], and 1–2 [c] viable colonies per petri plate at 53, 54, and 55 C, respectively); **B,** survival of mycelia. Vertical bars represent standard deviation.

DISCUSSION

Our results indicate that exposure of *M. piriformis* to high temperatures can kill its mycelia and sporangiospores. The two isolates tested showed a differential tolerance to elevated temperatures (Fig. 1). The isolate from Chile (CH) may have been adapted to the lower temperatures encountered in stone fruit orchards in Chile than in California. The mycelia of *M. piriformis*

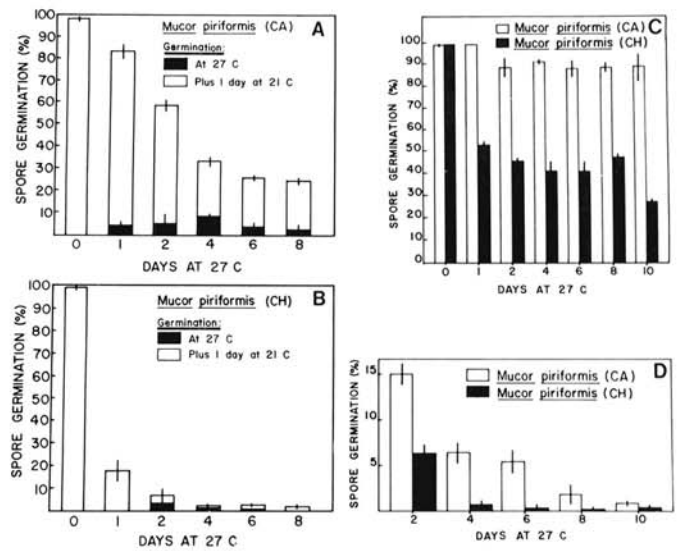

Fig. 2. Effect of 27 C temperature on germination and survival of sporangiospores of *Mucor piriformis* (California isolate [CA], Chile isolate [CH]). **A** and **B,** Sporangiospores on synthetic Mucor agar (SMA) incubated at 27 C for 1–8 days and one additional day at 21 C (producing normally and abnormally germinated sporangiospores). **C,** Sporangiospores incubated dry in vials at 27 C for 1–10 days, then plated on SMA, and incubated at 21 C for one additional day (producing sporangiospores germinated with normal and abnormal germ tubes). **D,** As in C above but only sporangiospores with normal germ tubes were recorded. Vertical bars represent standard deviation.

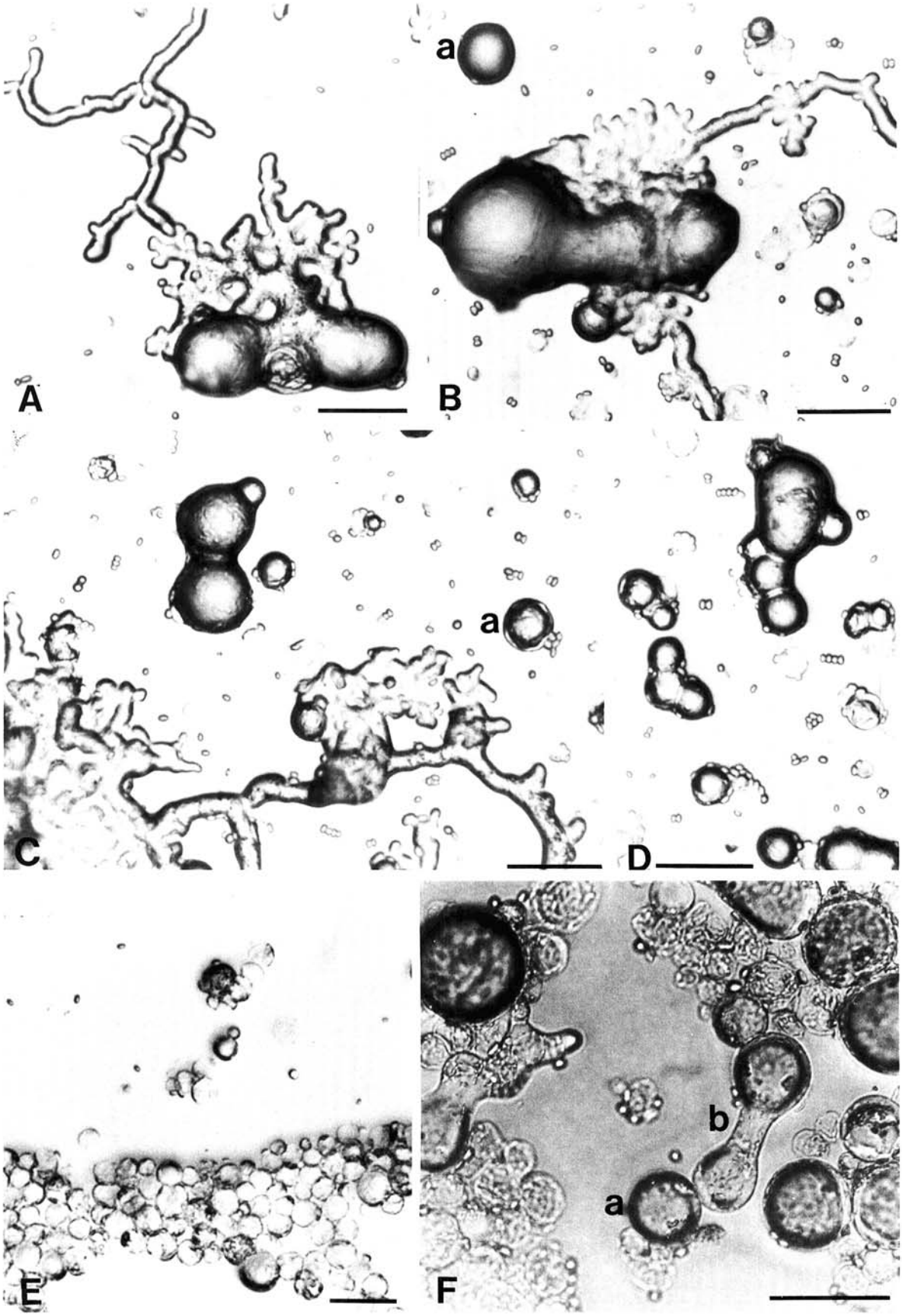

Fig. 3. Effect of 27 C temperature on the germination and growth of sporangiospores of *Mucor piriformis* incubated at 27 C for 2 days on synthetic Mucor agar. **A–D,** Abnormal and yeastlike growth of California (CA) isolate. **E** and **F,** Yeastlike growth of Chile (CH) isolate. a = swollen spores, b = a spore dividing by fission. Scale bar = 100 μm.

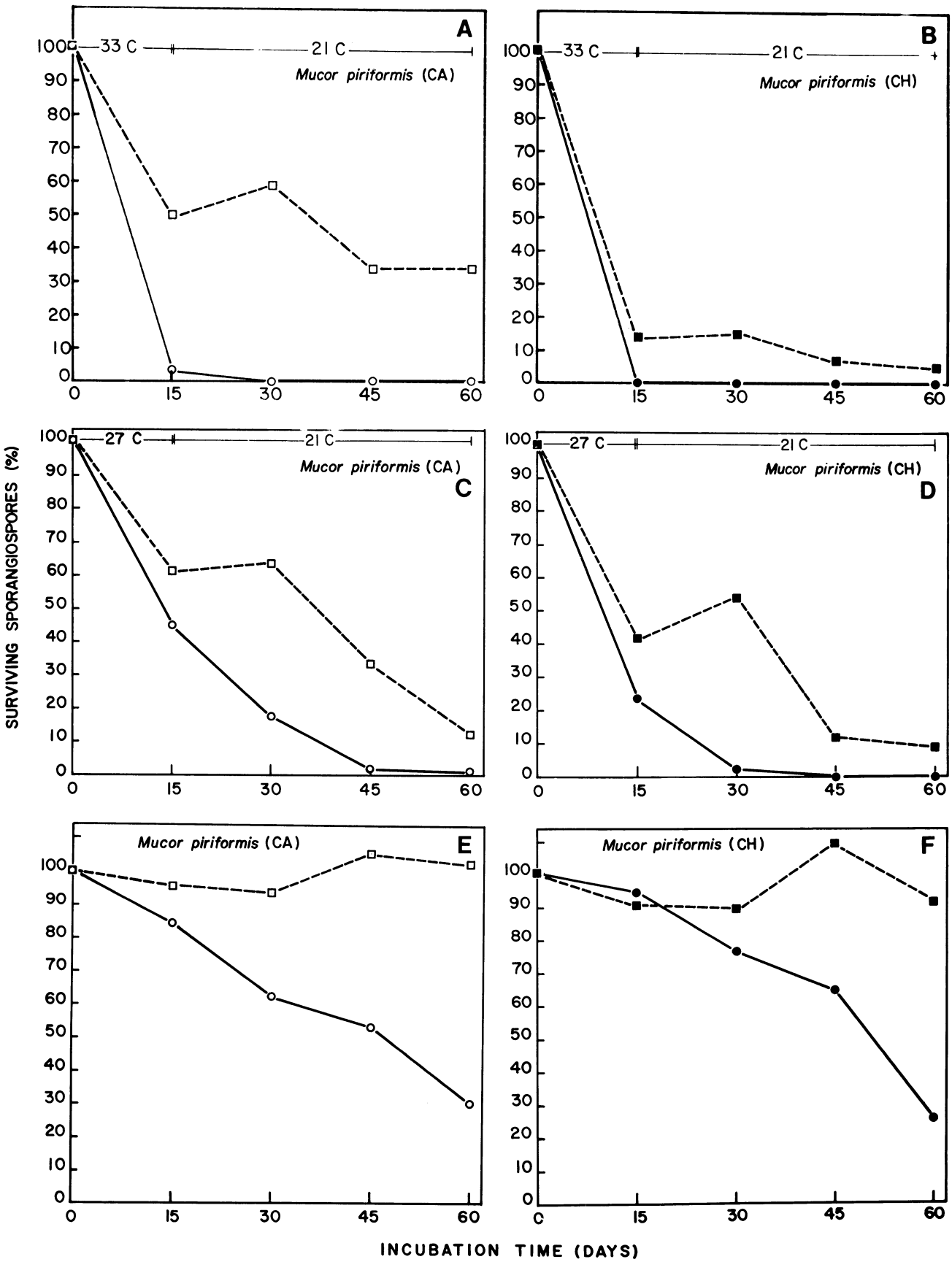

Fig. 4. Survival of sporangiospores of *Mucor piriformis* (California isolate [CA], Chile isolate [CH]) in a Hanford fine sandy loam soil as affected by predisposition temperature. Soil with sporangiospores was incubated at 33 C (**A** and **B**) or at 27 C (**C** and **D**) for 15 days and then at 21 C for up to 60 days. **E** and **F**, Continuous incubation at 21 C for up to 60 days. Soil moisture was maintained continuously at 20 g of water per 100 g of dry soil (−0.3 bar matric potential [wet, o or ●]), or soil initially at 20 g of water per 100 g of dry soil was allowed to dry to 1–2 g of water (−1,300 bars matric potential [dry, □ or ■]).

had a death point 9 C lower than that of the sporangiospores. Apparently, greater sensitivity to temperature fluctuations occurs in mycelia than in sporangiospores, which are probably better adapted to survival at higher temperatures. The ephemeral nature of fungal mycelia is well documented (14). Sporangiospores of *M. piriformis* were capable of surviving in soil for up to 1 yr, whereas mycelia survived for 1 or 3 wk in wet or dry soil, respectively (19). The results of the present study are in accordance with other studies that reported that fungal spores are more resistant than mycelia (14,15).

Smith et al (25) reported abnormal germination, cytoplasmic granulation, and wall breakdown of sporangiospores of *M. piriformis* held on PDA at 27 C for 1–10 days and then exposed to 18 C. Yeastlike growth occurred under temperature stress (27 or 0 C), depending on the isolate (25). Our data in general confirm these findings. Both isolates showed yeastlike growth with exposure to 27, whereas at 0 C the isolates always grew as hyphae (17). Temperature as well as specific nutritional factors have been reported to be the determinants for yeastlike growth in several fungi (11,24). In the numerous examples of thermally influenced dimorphism in filamentous fungi, the yeastlike phase is enhanced by elevated temperatures, whereas the filamentous form occurs at

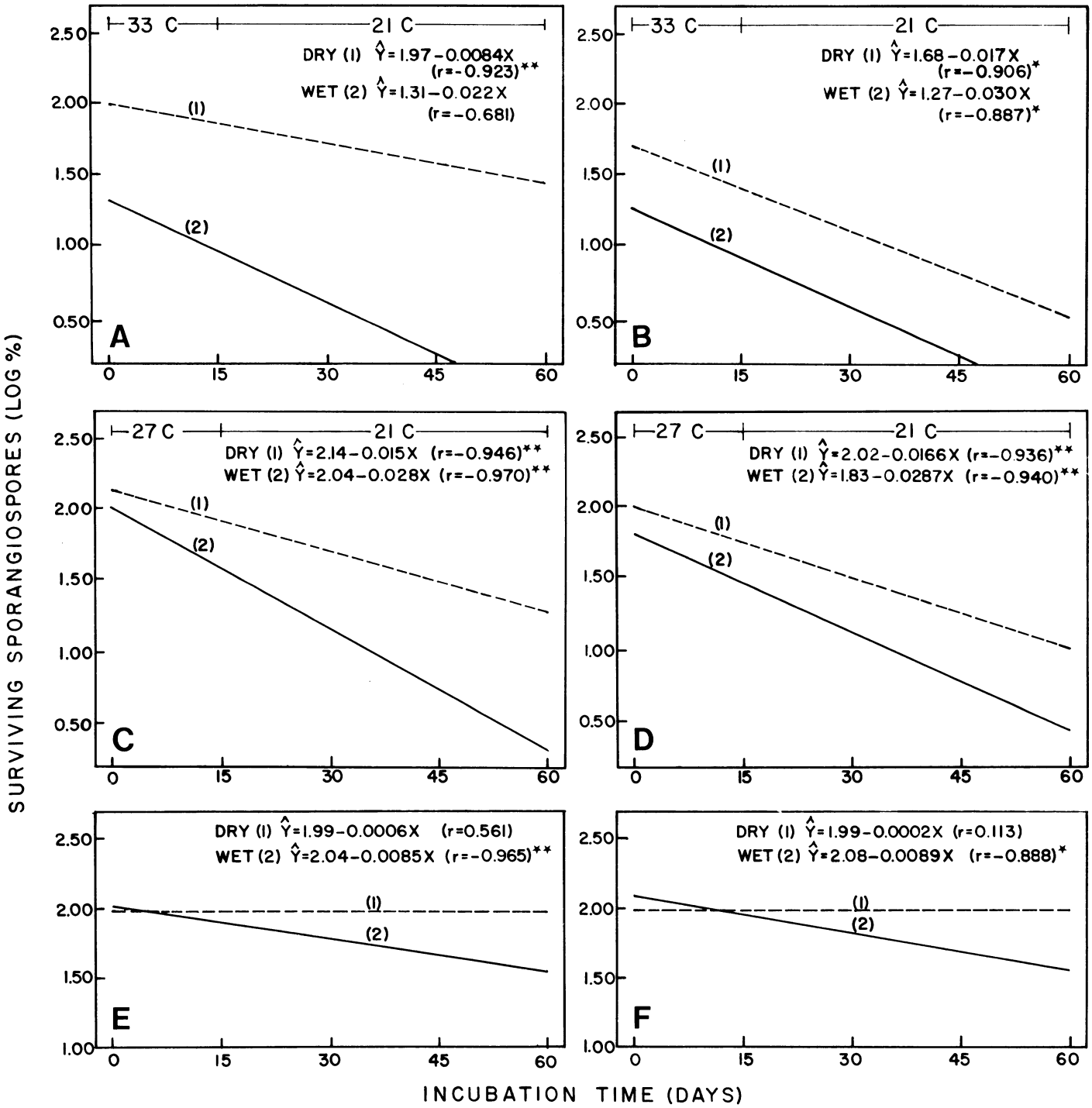

Fig. 5. Regression lines representing the survival of sporangiospores of *Mucor piriformis* (California isolate [CA], Chile isolate [CH]) in a Hanford fine sandy loam soil as affected by predisposition temperature. Predisposition at 33 C (**A** and **B**) or at 27 C (**C** and **D**) for 15 days and then at 21 C for up to 60 days. **E** and **F**, Continuous incubation at 21 C for 60 days. Soil initially at 20 g of water per 100 g of air-dried soil (−0.3 bars matric potential) was allowed to dry to 1–2 g of water per 100 g of dry soil (−1,300 bars matric potential [dry, line 1]); or soil moisture was maintained continuously at 20 g of water per 100 g of dry soil (wet, line 2). $\hat{Y}$ in the equations of the regression lines refers to the log of the percentage of surviving propagules and X to the incubation time in days (* = significance at $P = 0.05$, ** = significance at $P = 0.01$). A, C, and E = CA isolate; B, D, and F = CH isolate.

lower temperatures (4). For instance, growth of *Blastomyces dermatitis* Gilchrist & Stokes and *B. (Paracoccidioides) brasiliensis* (Splend.) Almeida at 37 C is yeastlike and at 25 C is hyphal (11). Both isolates of *M. piriformis* tested here grew yeastlike at 27 C and as hyphae at temperatures of 0–24 C (18). Therefore, elevated temperature is one of the environmental factors that induces yeastlike growth of sporangiospores of *M. piriformis*, and thus this fungus may be considered dimorphic. Increased temperature was one of the factors affecting dimorphism in *Mycotypha microspora* Fennel and *M. africana* Novak & Backus, fungi that also belong in the Mucorales (24).

Alterations of morphology and size of spores and developing germ tubes in response to elevated temperatures have been reported in several fungi (1–3). Spore swelling, thin-walled protrusions, and germ tube development occurred when spores of *Aspergillus niger* van Tieghem were incubated at 43 C for 24 hr (1). Vesicle swelling can be induced in the germ tubes of germinated urediniospores of several rust fungi in response to a variety of shock treatments, including elevated temperatures (12). Smith et al (25) reported that spores of *M. piriformis* on PDA increased nearly 6–8 times in size and germinated abnormally by producing swellings after 3 days of incubation at 27 C. We also observed swelling of sporangiospores of *M. piriformis* isolated from peaches and nectarines (Fig. 3B, C, and F) and abnormal germination of sporangiospores when they were incubated at 27 C for at least 2 days.

The significant ($P < 0.05$) interaction temperature × moisture indicates that the response of surviving propagules to moisture depends on whether or not propagules were preincubated at the high temperatures.

The survival pattern of sporangiospores with respect to temperature was similar in soil and on SMA. The decline in spore viability was greater in wet soil after 15 days of exposure to 33 C than in dry soil. Similarly, dry spores of *M. piriformis* maintained higher levels of germination than wet spores (Fig. 4). Nickerson et al (22) reported that wet sporangiospores of *Rhizopus stolonifer* (Ehr.:Fr.) Lind did not maintain viability compared with dry spores during storage at room temperature. Our results with *M. piriformis* parallel their findings. Thus, brief exposure to 27 C (i.e., for 2 days) resulted in a reduction in normal spore germination, suggesting that prolonged exposure to any temperature above 27 C may be detrimental. However, the decline of surviving sporangiospores in wet soil at 21 C indicates that additional factor(s) such as resident soil microflora or fungitoxic materials must be involved. The rapid decrease in viability of sporangiospores in soil under wet conditions, both when preincubated at 27 or 33 C for 15 days or continuously at 21 C, could in part be due to the loss of the mucilaginous water-soluble matrix. In species like *Mucor mucedo* L. :Fr. and *M. plasmaticus* van Tieghem and in *Phycomyces*, the sporangiospores are embedded in mucilage (30). This mucilaginous substance can be observed under the dissecting microscope by touching sporangia of *M. piriformis* with a needle. Nicholson and Moraes (21) demonstrated that removal of the mucilaginous matrix of conidia by water before storage significantly reduced the conidial viability of *Colletotrichum graminicola* (Ces.) Wils. during a 24-hr storage.

Fig. 6. Effect of heat treatment on infection of pear (Anjou) fruit by *Mucor piriformis* (California isolate). **A,** Fruits were dipped in water at 21 C for 30 min; **B,** fruits were dipped in a water bath maintained at 47 C for 30 min.

The concept of thermotherapy to control postharvest decay is not new, but several limitations have prevented the application of this system in packing sheds. In the Pacific Northwest, Spotts and Cervantes (27) were able to reduce fungal propagules in tank water by heating. Michailides and Spotts (20) showed that propagules of *M. piriformis* were present in soil scraped from harvest bins. In packinghouses, pear fruits are removed from field bins by immersion dumping; thus, soil and propagules of *M. piriformis* are continuously added to the dump water. It may be possible to maintain the water at 47 C to kill the propagules of *M. piriformis* or to prevent decay from propagules that have been introduced into fruit wounds in the field. Spotts and Chen (29) found that either wounded, heated, and then inoculated or wounded, inoculated, and then heated pear fruits had significantly lower decay caused by *M. piriformis* than did heated, wounded, and then inoculated pears, indicating that, apart from direct effects on the pathogen, heating may promote a wound-healing response. In our study, the fruits were wounded, inoculated, and then dipped in water of 47 C for 30 min, and percent infection of pear fruits was reduced. Since this short period of heating probably was not adequate for wound healing, the reduction of infection indicates that heating directly affected the pathogen propagules by reducing their viability. In addition, moist propagules, such as those in the dump water or brought in soil attached to the bottom of harvest bins (20), will decline faster than will dry propagules at high temperatures.

Our data indicate that high temperatures affect the propagules of *M. piriformis* by preventing sporangiospore germination or by killing both sporangiospores and mycelia. In the field, survival of sporangiospores of *M. piriformis*, which are long-term survival structures (19), depends largely on temperature and moisture stresses. Temperatures of 27 and 33 C, which are common in California peach and nectarine orchard soils during summer (19), may kill the sporangiospores or prevent them from causing fruit infection. This may explain the sporadic occurrence of Mucor rot in the frequently irrigated stone fruit orchards in California. Fruits on the ground or standing in the irrigation water exposed to the sun may not be infected by propagules of *M. piriformis*. Frequently, these fruits are infected and decayed by fungi such as *R. stolonifer, Gilbertella persicaria* (Eddy) Hesseltine, or *Monilinia fructicola* (Wint.) Honey. Therefore, no additional inoculum (sporangiospores) of *M. piriformis* would be produced to add to the continuously declining levels of propagules in soil. In addition, during late fall and winter, although soil temperatures are favorable (low) for survival and development of *M. piriformis*, there are no fruits on the ground for infection and massive production of sporangiospores. In contrast, in the Pacific Northwest, significant increase of sporangiospore inoculum of *M. piriformis* occurs in late fall when conditions for infection and sporulation of the fungus are favorable and concurrently, pear fruits on the orchard floor are abundant (20). In the Hood River Valley of Oregon, levels of sporangiospores of *M. piriformis* in soils sampled from pear orchards are higher (20,28) than those in soils obtained from California peach orchards (T. J. Michailides, *unpublished*).

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