Survival of *Mucor piriformis* on Artificially Inoculated Fruit Endocarps of *Prunus persica*

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ABSTRACT

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In laboratory tests, sporangiospores of *Mucor piriformis*, isolates CA from California and CH from Chile, added to peach and nectarine endocarps and then partially buried in wet (−30 kPa matric potential) and dry (-1.3×10^5 kPa matric potential) soil survived longer at 0 and 10 C than at 27 and 33 C. More sporangiospores survived on endocarps buried in dry than in wet soil. Survival of sporangiospores declined over time in a pattern described by polynomial equations in wet soil at all temperatures and in dry soil only at 33 C. Both isolates grew and sporulated on autoclaved endocarps incubated in moisture chambers at 0, 10, and 21 C, but not on those incubated at 27 or 33 C. An agar medium made from endocarp washings favored germination of sporangiospores and significantly increased growth and sporulation of *M. piriformis* compared with plain water agar. Mummified peaches and nectarines artificially inoculated with isolates CA and CH of *M. piriformis* were exposed to winter and summer soil conditions in the field. Recovery of the fungus during fall and winter months (October to March) was 100%. Approximately 5 mo from the time the endocarps were buried in the soil, decline of propagules on the endocarps lacking fruit tissues was exponential for both isolates, with more propagules of the CA isolate surviving than the propagules of the CH isolate after 1 yr. Chlamydosporelike structures were found in hyphae, and sporangiophores developed in decayed mesocarp tissues. More than 75% of the colonies of *M. piriformis* that grew from propagules on endocarps originated from sporangiospores.

Additional keywords: postharvest pathogen, psychrophilic fungus.

Mucor piriformis Fischer was not considered to be part of the complex of postharvest pathogens of stone fruits in California until 1977 when it caused serious losses during transit of fresh-market peaches (*Prunus persica* (L.) Batsch) shipped from California to the eastern United States and in nectarines (*P. persica* var. *nectarina* (Aiton) Maxim.) shipped from Chile to California (13). Since then, however, Mucor rot has not caused significant losses of peaches and nectarines in California, probably because of climatic conditions, but postharvest losses caused by *M. piriformis* can become a serious problem (1,23). There is little published information on Mucor rot of peaches and nectarines in California (13,23).

Survival of sporangiospores of *M. piriformis* in soil is temperature and moisture dependent and can exceed 1 yr if the temperature remains below 27 C in dry soil (17). However, in the major peach-growing areas of California, temperatures usually are higher than 27 C, and survival of propagules of *M. piriformis* can be affected (17). Thus, propagules of *M. piriformis* are not common in orchard soils of the San Joaquin Valley in California but are in soils of pear orchards in the Hood River Valley in Oregon (24).

Survival of many fungi depends on plant debris on the soil (12) or buried in soil (2). Dobson et al (5) found that addition of pear fruits to a soil resulted in an increase of propagules of *M. piriformis* between September and January. Michailides and Ogawa (18) showed that *M. piriformis* failed to colonize nonautoclaved soil or autoclaved soil amended with soil microorganisms; however, in soils amended with organic matter and soil microorganisms, the fungus grew and sporulated. Leaves of peach and of common weeds also supported saprophytic growth and sporulation of *M. piriformis* (18). In addition, glucose amendments favored germination of sporangiospores of *M. piriformis* only in soil without antagonistic microorganisms and not in nonautoclaved soil (18). Dobson and Spotts (6), however, used nonautoclaved soils from pear orchards and showed that the fungus colonized these soils only at 5–15 C and not at 20–25 C.

Inoculations in the field have indicated that peach or nectarine fruits infected with *M. piriformis* form mummies that can give rise to abundant sporangia depending on environmental conditions. Survival of *M. piriformis* on intact mummified peaches buried 5 cm deep in orchard soils ranged from 9 to 19 mo and was dependent on both isolate and temperature (17). Fleshy mesocarp tissues of peaches and nectarines in contact with soil disintegrate within a 6-mo period; hence, inoculum potential of *M. piriformis* the following season became minimal (17). Under these adverse edaphic conditions of high soil temperatures and limited fruit tissue substrates, any situation or condition that aids survival may play a significant role in the life cycle of the fungus. The endocarps of peach and nectarine fruits on or partially buried in orchard soils can persist for 1 or more years and could serve as sites of survival of *M. piriformis*. Limited sporulation of the fungus was observed among the ridges and in the cavity of nectarine endocarps in a commercial orchard in Parlier, CA (Michailides, *unpublished*).

Because registered fungicides are ineffective against *M. piriformis*, control measures aimed at reducing inoculum must be based on a better understanding of the pathogen's life cycle. To date, no information has been published regarding the role of peach and nectarine endocarps on the behavior and survival of propagules of *M. piriformis*. Thus, this research was initiated to determine if endocarps of mummified peach and nectarine fruit could serve as potential sites for survival and growth and sporulation of *M. piriformis*.

MATERIALS AND METHODS

Isolates. Two isolates of *M. piriformis*, one from a decayed peach in California (isolate CA, American Type Culture Collection 52555) and another from a decayed nectarine shipped to California from Chile (isolate CH, American Type Culture Collection 52554), were used throughout this study. In previous studies, these isolates differed in sensitivity to temperature (14,19).

Survival of sporangiospores on peach endocarps buried in soil and incubated at various temperatures. Only sporangiospores were used in laboratory experiments because they are the long-term survival structures of *M. piriformis* (15,17). Endocarps were removed from freestone Fay Elberta peaches, washed free of fleshy mesocarp tissues, autoclaved (120 C and 104 kPa pressure for 30 min), and air dried on a laboratory bench for 10 days. Sporangiospores for each CA and CH isolate were produced by wound inoculating peach fruit with 20 μl of a suspension containing 1×10^5 sporangiospores/ml, prepared from a 6-day-old potato-dextrose agar (PDA) culture of each isolate of *M. piriformis*. Inoculated fruits were held at 21 C for 20 days. Then sporangia were washed off with 135 ml of water per fruit, and the resultant spore suspension was adjusted to 2.6×10^6 sporangiospores/ml. Endocarps were dipped in the spore suspension, agitated for 2 min, and placed over wire screens to drain and dry.

A Reiff loam soil (20–25% clay, 40–45% silt, and 30–40% sand) from an experimental peach orchard at the University of California, Davis, was screened through a 5.6-mm-mesh sieve to eliminate large debris, air dried at 22 ± 1 C on a laboratory bench for 4–5 days, and distributed in 100-g portions into deep (10 × 2.5 cm) petri dishes. In half of the dishes, 20 ml of sterile distilled water was added and mixed with the soil, resulting in −30 kPa matric potential. Soil in remaining dishes without water added had a matric potential of -1.3×10^5 kPa. Matric potential of dry soil was determined with a thermocouple psychrometer (isopiestic technique [8]). Water content at field capacity was 31.5%, as determined with ceramic pressure plates (Soil Moisture Equipment Co., Santa Barbara, CA). Three endocarps contaminated with sporangiospores were partially buried (approximately 25% of their volume exposed) in each petri dish containing either wet (20 g of water/100 g of dry soil) or dry soil (1–2 g of water/100 g of dry soil). All dishes were wrapped in two plastic bags to prevent moisture loss and then incubated at 0, 10, 21, 27, and 33 C. A 2 (soil moistures) × 2 (isolates) × 5 (temperatures) × 5 (times) factorial experiment in a split-split-plot design with three replicates (three petri dishes each containing three endocarps) was used.

Endocarps were assayed for viable spores after 0, 5, 18, 60, and 120 days. Adhering soil was removed with forceps, and the endocarps were examined under a dissecting microscope for morphological structures of *M. piriformis*. To determine survival of sporangiospores, the three endocarps from each plate were placed in a 250-ml Erlenmeyer flask containing 100 ml of sterile distilled water and shaken at 200 rpm for 1 hr. The water suspensions were diluted to 10^{-3} or 10^{-4}, and 100 μl was spread evenly on the surface of each of 10 dishes of PDA acidified to pH 3.5 ± 0.1 (APDA). After 24–26 hr of incubation at 21 C, colonies of *M. piriformis* were counted by viewing the dishes through transmitted light. Petri dishes then were placed at 0–1 C for an additional 4–5 days, and colonies were counted again.

The experiment was repeated, and results are the average of the two tests. Average numbers of surviving propagules for each isolate at each sampling time were expressed in percentages of the initial density of sporangiospores and transformed to $\log_e$ (4). Survival-over-time data at each temperature were analyzed by regression (Statistical Analysis Systems, SAS Institute, Inc., Cary, NC).

Saprophytic colonization of peach and nectarine endocarps in absence of soil. An experiment was designed to determine the saprophytic development of isolates CA and CH of *M. piriformis* on peach and nectarine endocarps in the absence of soil. Autoclaved endocarps were dipped for 10 min in a suspension of 3×10^5 sporangiospores/ml, agitated with a glass rod, and placed over wire screens to drain. Four endocarps were placed in each of three petri dishes (10 × 2.5 cm) containing two Whatman No. 1 filter papers premoistened with 3 ml of sterile distilled water in the bottom and two more attached to the inner surface of the petri dish cover. To avoid moisture loss, dishes were sealed with Parafilm (American Can Company, Dixie/Marathon, Greenwich, CT), placed in plastic bags, and incubated at 0, 10, 21, 27, and 33 C for up to 40 days. A 5 (temperatures) × 2 (isolates) factorial experiment in a split-plot design with three replicate dishes each containing four endocarps was used. This experiment was repeated once.

Fungal colonization of the endocarps was determined after 10–13 days, and sporangiospore population densities were determined after 40 days. The latter determination was accomplished by placing four endocarps per replicate per temperature treatment in a 250-ml Erlenmeyer flask containing 50 ml of sterile distilled water and shaken at 200 rpm for 2 hr. After appropriate dilutions of the resultant suspension, 100 μl was plated on each of five APDA dishes per replicate and incubated at 21 C for 22–24 hr. Sporangiospores produced per endocarp were calculated from colonies of *M. piriformis* counted per dish. Data for the two isolates for each temperature were compared with a pairwise *t*-test.

Effect of endocarp washings on growth and sporulation of *M. piriformis*. Three autoclaved peach and nectarine endocarps were placed in each of three 250-ml Erlenmeyer flasks containing 50 ml of sterile distilled water and shaken at 200 rpm for 2 hr. The endocarp extract was collected and used to prepare sporangiospore suspensions (3×10^4 spores/ml) from 2-day-old cultures of *M. piriformis* (isolates CA and CH). The control was a spore suspension (3×10^4 spores/ml) prepared with sterile distilled water. One-half milliliter of each suspension for each CA and CH isolate was plated on four replicate water-agar (WA) dishes in a two-way factorial experiment in a split-plot design. Dishes were incubated at 21 C for 18 hr, and sporangiospore germination rates were determined. After further incubation for an additional 48 hr, 20 random colonies were examined with a compound microscope (×80), and the numbers of sporangiophores with sporangia were recorded for each colony. In addition, the concentration of sporangiospores per dish was determined by adding 10 ml of sterile distilled water to each dish, counting the released sporangiospores with the aid of a hemacytometer, and subtracting the sporangiospores initially seeded per dish.

In another experiment, an endocarp-washings agar (EWA) consisting of 15 g of agar/1 L of endocarp washings was prepared. A 5-mm-diameter mycelial disk taken from the margin of a 2-day-old culture of each isolate of *M. piriformis* grown on APDA was placed in the center of four petri dishes containing WA or EWA and incubated at 21 C for 5 days. Radial growth of the fungus was measured daily for 4 days, and the number of sporangiospores produced per colony after 5 days of incubation at 21 C was determined with the aid of a hemacytometer. Both experiments were repeated, and results represent the average of the two experiments.

Survival of *M. piriformis* on decayed peach and nectarine under prolonged field exposure. Two sets each of 24 freestone fruits of peach (cultivar Halloween) and of nectarine (cultivar Le Grand) were surface disinfested with 0.08% NaOCl for 3 min and wound inoculated with isolate CA or CH (50 μl per wound of a 1.2×10^5 sporangiospores/ml suspension). Each set of 24 fruits (12 peach and 12 nectarine) per isolate was closely spaced on the soil beneath two separate peach trees on 15 October 1981. The 24 fruits of each set were collectively housed in a wooden-sided frame (65 × 45 × 15 cm). The frame had no bottom, and its top was covered with metal screen (1-mm^2 mesh) to protect the fruit from rodents and birds. Fruits decayed within 1 wk, and the fleshy mesocarp tissue disintegrated slowly over winter. At 1-mo intervals for 5 mo, five random samples (approximately 0.2 cm^3) of decayed (or mummified) fruit mesocarp tissues were taken with forceps. Samples were transferred to APDA slants, incubated at 0–1 C for 15–20 days, and rated for fungal growth. In addition, slide mounts of decayed tissues were examined with a compound microscope (×200 or ×320) for specialized survival structures of the fungus.

Beginning on 6 March 1982 when mesocarp tissue appeared completely decomposed, endocarps (now in intimate contact with the soil) were assayed for propagules of *M. piriformis*. At subsequent intervals of 31, 54, 85, 107, 147, and 200 days, three endocarps of peach or nectarine per replicate per isolate were collected from each fruit set and assayed as described previously.

The last sampling was done on 16 October 1982 (365 days from the time of inoculation of the fruit in the field). This experiment was repeated in the 1982-1983 season.

The number of propagules recovered was averaged for each isolate at each sampling time and was expressed as a percentage of the initial density of sporangiospores recovered in the March sampling. Percentages were transformed to $\log_e$ for analysis (4). Log_e of percentages of propagules recovered were regressed on time (days), and comparisons of the two isolates were made with analysis of variance (Statistical Analysis Systems, SAS Institute). Soil temperatures were measured with a two-point thermograph (Model 4020, Weathertronics, Inc., Sacramento, CA).

Nature of surviving propagules on endocarps. Because hyphae and sporangiophores developed on the decayed tissue of inoculated fruit and both were present on the endocarps in the field, the nature of propagule units that survived on the endocarps after 1 yr in the field was determined. Eighty-five colonies of *M. piriformis* randomly selected from the dilution dishes that had been incubated at 21 C for 24 hr were observed individually under a compound microscope (×80) to determine the nature of the propagule from which the colony originated. The number of germ tubes emerging from individual propagules also was recorded and compared with the germ tubes of germinated sporangiospores of *M. piriformis* plated on APDA.

RESULTS

Survival of *M. piriformis* on peach endocarps in the laboratory. Sporangiospores of isolate CA survived at significantly ($P < 0.01$) higher rates than those of isolate CH at temperatures from 0 to 27 C. At 33 C, both isolates declined at similar rates. Overall, however, both isolates showed similar trends; hence, only results obtained for the CA isolate are illustrated (Fig. 1A–J).

Under wet conditions, the survival rate of propagules declined in a way best described by polynomial (quadratic) equations. In general, the rate of decline in sporangiospore viability was slower at 0–21 C (Fig. 1A, B, and C) than at 27 and 33 C (Fig. 1D and E). Except at 0 C, survival rates in wet versus dry conditions were significantly different at all other temperatures tested. For wet soils, all correlation coefficients (r) were significant ($P < 0.05$), whereas for the dry soil, r was significant only at 33 C (Fig. 1).

Propagule decline in dry soil was much slower (smaller negative slope values [Fig. 1F–J]) than in wet soil (larger negative slopes [Fig. 1A–E]) at all temperatures except 0 C. At 0 and 10 C in wet soil, the number of propagules recovered increased after 18–60 days and 120 days, respectively, indicated by the positive slopes in the equations (Fig. 1A and B). Microscopic examination of sampled endocarps revealed that the fungus grew and produced sporangia that were smaller than normal (13). In wet soil, sporangiospores of the CA isolate survived on endocarps for at least 120 days at 0, 10, and 21 C, for 60 days at 27 C, and for 5 days at 33 C. In dry soil, propagules were viable after 120 days at 0, 10, 21, and 27 C, and for 60 days at 33 C.

Saprophytic colonization of autoclaved peach and nectarine endocarps in the absence of soil. Both isolates colonized the surface of endocarps incubated at 0, 10, and 21 C after 10–13 days (Table 1 and Fig. 2) but not at 27 and 33 C incubated for 40 days. The CA isolate produced significantly larger numbers of sporangiospores than did the CH isolate at 0–21 C ($P \leq 0.01$). Both isolates produced more sporangiospores at 10 and 21 C than at 0 C ($P \leq 0.01$) (Table 1).

Effects of endocarp washings on *M. piriformis*. After 18 hr at 21 C, endocarp washings induced 48–49% sporangiospore germination. In comparison, only 0.5–0.75% germination occurred in water controls. Ninety-five percent of germinated sporangiospores produced a single, simple (14%), or sympodially branched (81%) sporangiophore; 10% of these sporangiophores originated directly from the germinated sporangiospores, and the remainder originated from hyphae. Numbers of produced sporangiospores ranged from 1.4×10^5 to 1.8×10^5 or 0 on WA dishes seeded with sporangiospore suspensions prepared in endocarp washings or distilled water, respectively.

The isolates grew faster on EWA (Fig. 3A) where sporulation was extensive. On WA, sporulation was confined to the APDA disk. The isolates produced 9.9×10^6 (CA) and 4.1×10^6 (CH) sporangiospores on EWA and 1.04×10^6 (CA) and 0.54×10^6 (CH) sporangiospores per dish on WA (Fig. 3B).

Survival of *M. piriformis* on decayed peach and nectarine fruits under prolonged field exposure. Both isolates were readily isolated on APDA slants during the first 5 mo of sampling (fall and winter). Microscopic examination of decayed and mummified fruit tissues revealed occasional chlamydosporelike structures in empty hyphae (Fig. 4A–C) and sporangiophores (Fig. 4D) for both isolates. Some hyphae showed retracted or fragmented protoplasts. In addition, the CH isolate had darkened columellae, with parts of the sporangiophore attached to them (Fig. 4E). Germination of chlamydosporelike structures was seen under field and culture conditions (Fig. 5A and B). Some of the germinated chlamydosporelike structures produced a single sporangiophore (Fig. 5A and B [a]) bearing a small sporangium (Fig. 5A and B [b]). Germinated chlamydosporelike structures usually lacked protoplasmic accumulations (Fig. 5A and B [c]). In a number of samples, abundant chlamydospores of *M. racemosus* Fresenius also were present.

Arithmetic plots of percentage of surviving propagules on endocarps with sampling time were curvilinear (Fig. 6A–D). In both years, survival rates of isolates of *M. piriformis* were similar. When percentages of surviving propagules were transformed to natural logarithms and plotted against sampling time expressed in days, a straight-line, negative relationship was obtained (Fig. 6A–D). All correlation coefficients (r) were highly significant. A comparison of the slopes of the lines of survivors (3) for the two isolates showed that they differed significantly from each other ($P < 0.01$) in 1981–1982, indicating that the propagules of isolate CA declined more slowly ($P < 0.01$) than those of isolate CH during that period (Fig. 6A and B). However, the rates of propagule decline (slopes of the lines of survivors) of the two isolates in 1982–1983 did not differ significantly ($P > 0.05$) (Fig. 6C and D). About 2–4% of the original population level (1.92×10^3 propagules/endocarp) of isolate CA could be recovered after 200 days (Fig. 6A). One year after inoculation of the fruit in the orchard, 133 propagules/endocarp of isolate CA were recovered, whereas no propagules of isolate CH were recovered (Fig. 7A). Isolate CH was recovered 147 days after 6 March (8–9 propagules/endocarp), but not after 200 days (Fig. 6B and D). When dilution dishes were kept at room temperature (22 ± 1 C) for 2 days, an average of 15 colonies of *M. racemosus* developed per dish.

The weekly mean and maximum soil temperatures recorded in the experimental plots under the peach trees ranged from 10 to 15 C during winter and from 23 to 28 C during spring and summer, respectively.

Nature of surviving propagules on endocarps. Because the developing fungus decayed the fruit, it is probable that both hyphae and sporangiospores initially were present on the endocarps. However, microscopic examination of 85 random colonies in dilution dishes on 6 March and on subsequent dates revealed that at least 75% of the colonies originated from sporangiospores producing 1–3 germ tubes (Fig. 7B–D). The origins of the remaining 25% of the colonies were covered with soil.

DISCUSSION

The survival of sporangiospores of *M. piriformis* on stone fruit endocarps partially buried in soil was dependent on temperature, moisture, and fungus isolate (Fig. 1), which agrees with results of a previous study in which sporangiospores were mixed with soil (17). In that study, the numbers of propagules declined over time in an exponential fashion; on endocarps, the numbers of propagules declined slower, probably because of the presence of nutrients on endocarps. With endocarps incubated at 0–10 C in wet soil, propagules of isolate CA increased during 18–60 days of the experiments via development of sporangia by the fungus.

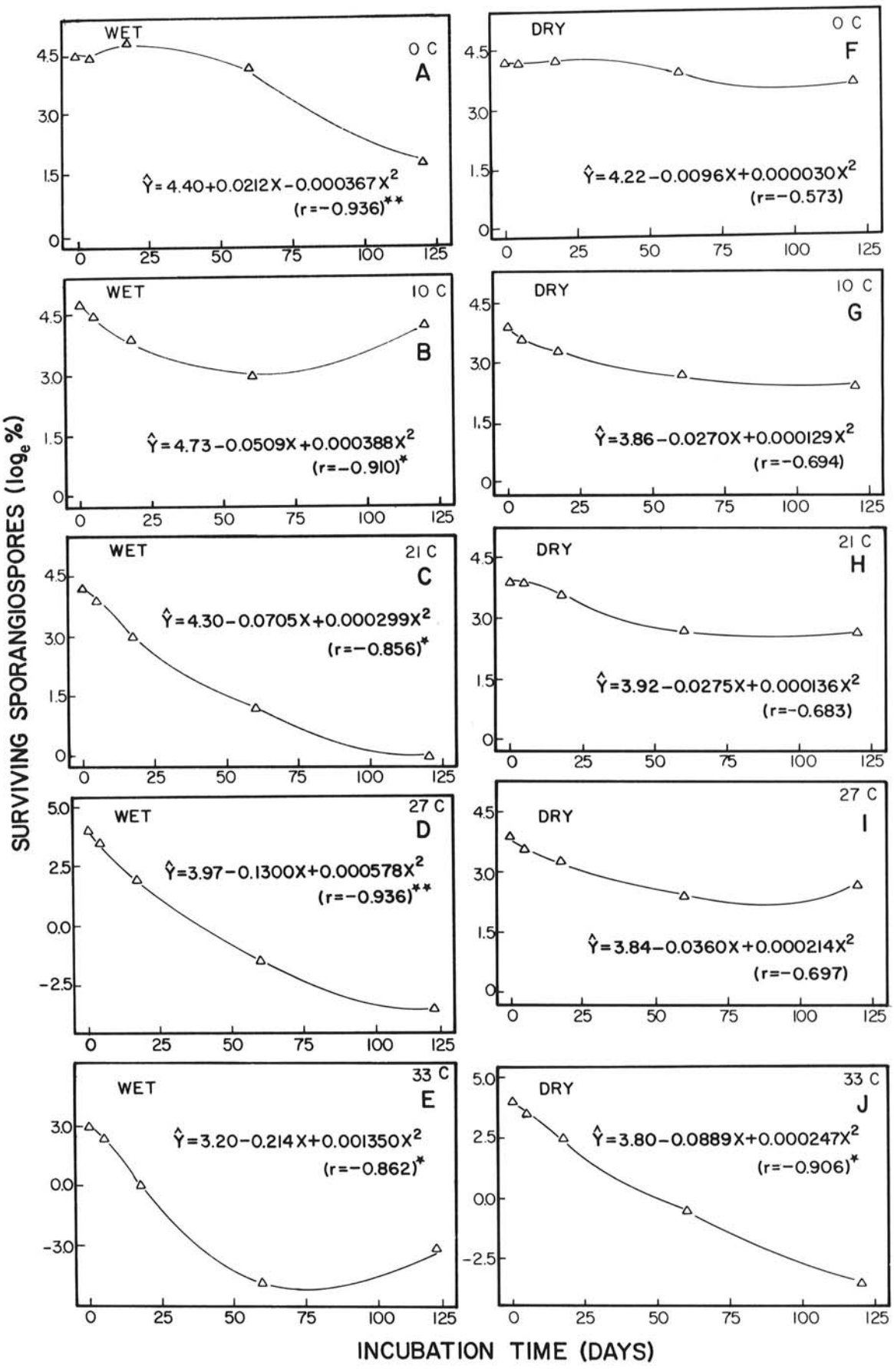

Fig. 1. Regression lines for sporangiospore survival of *Mucor piriformis* (isolate CA from California) on peach endocarps after 120 days at five constant temperatures and two soil moisture levels in a Reiff loam soil. Temperature was maintained at 0 C (**A and F**); 10 C (**B and G**); 21 C (**C and H**); 27 C (**D and I**); and 33 C (**E and J**). Soil moisture was maintained at −30 kPa matric potential (**A, B, C, D, and E**) or at −1.3 × 10^5 kPa matric potential (**F, G, H, I, and J**). Y in regression equations is $\log_e$ of the percentage of surviving sporangiospores, and X is incubation time in days. * = significance at $P = 0.05$; ** = significance at $P = 0.01$.

In addition, in culture, nutrients derived from endocarps supported germination, growth, and sporulation of the fungus (Table 1, Figs. 2 and 3).

In general, cold and dry conditions contribute to long-term survival of fungi and other microbes (9). Sporangiospores of *M. piriformis* on endocarps followed this pattern by surviving longer on endocarps buried in dry soil than on those buried in wet soil.

Sporangiospores on inoculated peaches buried 5 cm deep in soil survived 9–19 mo, depending on the isolate of *M. piriformis* and environmental conditions at the burial site (17). In the present

TABLE 1. Growth and sporulation of *Mucor piriformis* (isolates CA from California and CH from Chile) on the surface of peach and nectarine endocarps[a] at various temperatures for 40 days

Temperature	Presence of sporangia after 10–13 days		Sporangiospores/endocarp ($\times 10^3$) after 40 days[b]	
	CA	CH	CA	CH
0	+	+	33.4**	5.6
10	+	+	1,222.5**	716.3
21	+	+	1,350.0**	765.0
27	−	−	0	0
33	−	−	0	0

[a] Endocarps were dipped in a suspension of 3×10^5 sporangiospores of *M. piriformis* per milliliter for 10 min, drained, placed in moisture dishes, and incubated at the different temperatures for 40 days.

[b] Number of sporangiospores was determined from colonies developed on five replicate acidified potato-dextrose agar dishes incubated at 21 C for 22–24 hr. At 0, 10, and 21 C, numbers of sporangiospores of isolate CA followed by ** differ significantly at $P = 0.01$ from those of isolate CH according to a pairwise *t*-test.

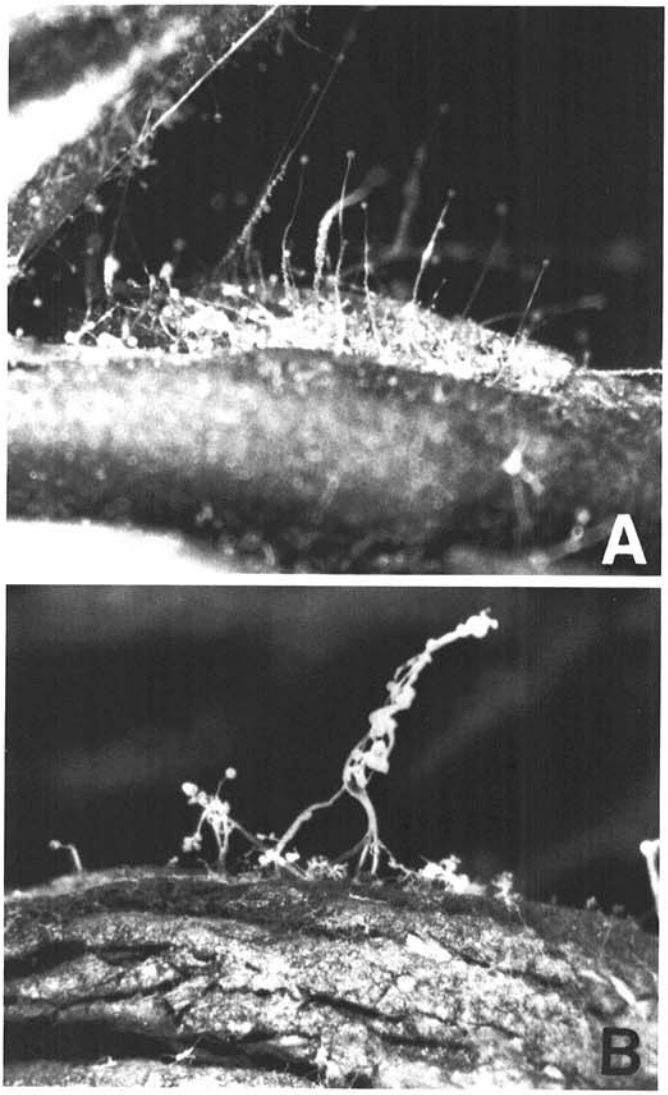

Fig. 2. Growth and sporulation of *Mucor piriformis* on peach and nectarine endocarps in the absence of mesocarp tissues. **A,** Isolate CA from California. **B,** Isolate CH from Chile.

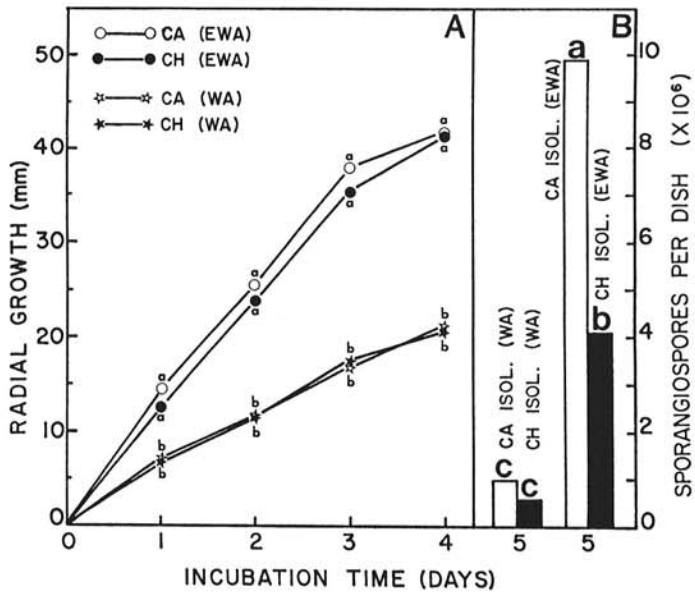

Fig. 3. Growth and sporulation of *Mucor piriformis* (isolates CA from California and CH from Chile) on water agar (WA) or endocarp-washings agar (EWA) incubated at 21 C. **A,** Radial mycelial growth after 4 days. The linear regression equations are as follows: CA (EWA): $Y = 2.54 + 10.63X$ ($r = 0.986$)**; CH (EWA): $Y = 1.43 + 10.57X$ ($r = 0.995$)**; CA (WA): $Y = 0.93 + 5.16X$ ($r = 0.995$)**; and CH (WA): $Y = 0.94 + 5.13X$ ($r = 0.994$)** (** = significance at $P = 0.01$). **B,** Numbers of sporangiospores produced per dish after 5 days. Points of radial growth for the same time and numbers of sporangiospores with different letters differ significantly according to Duncan's multiple range test ($P < 0.05$).

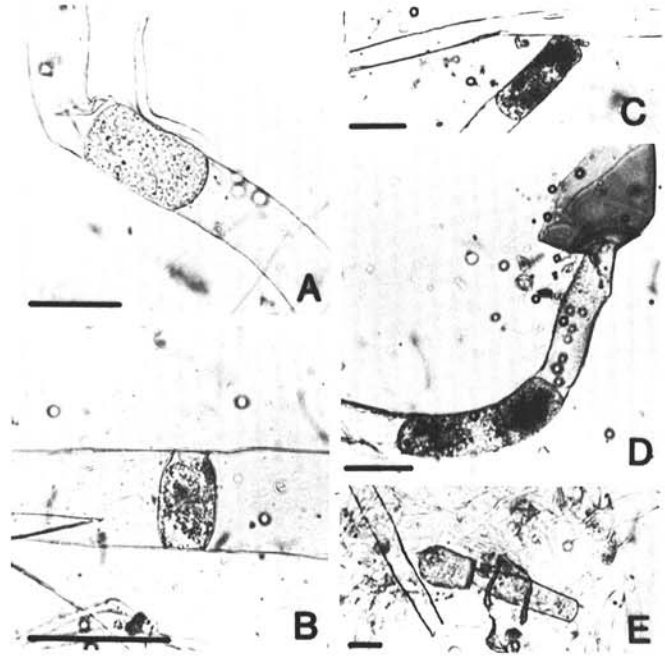

Fig. 4. Chlamydosporelike structures developed in hyphae and sporangiophores of *Mucor piriformis* on infected peach and nectarine fruits partially buried in soil. **A, B, and C,** Chlamydosporelike structure of isolate CA (from California) in hyphae. **D,** Chlamydosporelike structure in a sporangiophore of isolate CH (from Chile). **E,** Accumulation of protoplasts in the columella and upper part of a sporangiophore of isolate CH. (Bar = 50 μm).

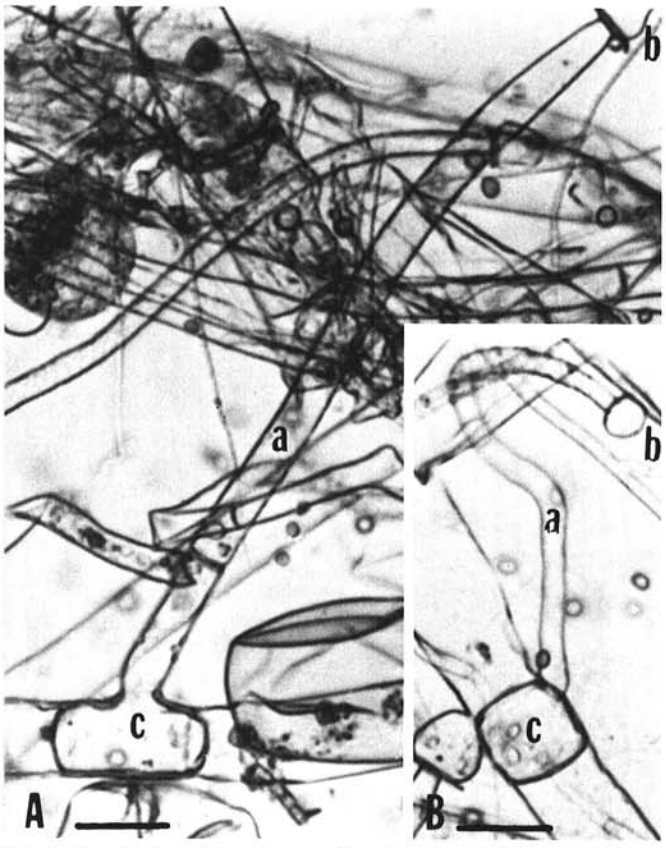

Fig. 5. Germinated chlamydosporelike structure of *Mucor piriformis* recovered from decayed tissues of a peach **(A)** and a nectarine **(B)** left on the orchard ground for 3–4 mo. a = sporangiophore, b = columella of produced small sporangium, c = chlamydosporelike structures lacking protoplasmic accumulations. Bar = 50 μm.

study, I demonstrated that propagule viability on peach and nectarine endocarps in the absence of mesocarp tissue showed an exponential decline similar to that of propagules mixed with soil and buried in a peach orchard (17). Both isolates overwintered successfully on infected mummified peaches and nectarines on the ground. The development of chlamydosporelike structures (Fig. 4) may have helped the fungus survive in soil under the adverse conditions. In response to unfavorable conditions, some fungi, such as *M. racemosus* or *Fusarium* spp., show modification of hyphae by the production of thick-walled chlamydospores (11). Park (22) reported that soil stimulates the production of chlamydospores in fungal hyphae of *F. oxysporum* Schlechtend.:Fr., and Wilhelm (25) found that conidia of *Verticillium albo-atrum* Reinke & Berthier may become transformed into either chlamydospores or microsclerotia. Similarly, chlamydosporelike structures were observed in hyphae of the CA and CH isolates buried in soil (15), on artificially inoculated peach and nectarine mesocarps, and in old cultures of *M. piriformis* (Michailides, *unpublished*). In this study, chlamydosporelike structures also were found in the sporangiophores (Fig. 4D). Development of chlamydosporelike structures in *M. piriformis* is the result of retracted protoplasts in hyphae and sporangiophores, characteristics that also were observed previously when mycelia were buried in wet soil (17). Accumulation of protoplasts in columellae also was noticed; such protoplasmic accumulations led to columellar germinations and production of sporangia (13,16).

According to earlier reports, *M. piriformis* does not produce true chlamydospores in culture (7,27); however, production of chlamydosporelike structures in nature may play a role in the survival of the species. Sporangia produced from germinated chlamydosporelike structures with the consumption of protoplasmic accumulation (Fig. 5A and B) might serve as an inoculum source of the fungus in orchard soils. Although zygospores are present in the life cycle of *M. piriformis* (20), they rarely are found in nature (21), and their role in survival of the fungus is still unknown. *M. racemosus* is a common soil, mucoraceous

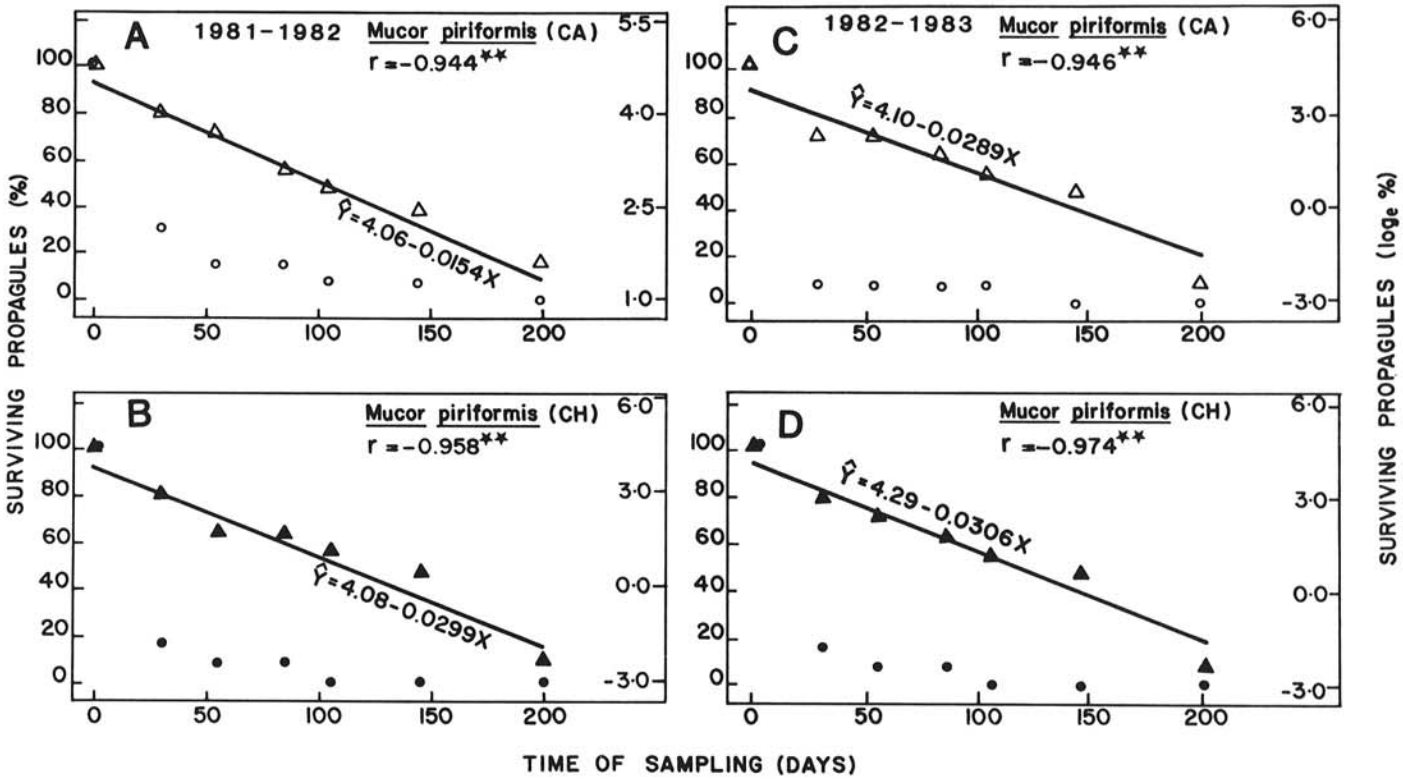

Fig. 6. Regression lines for the survival of propagules of *Mucor piriformis* (isolates CA from California and CH from Chile) on peach and nectarine endocarps partially buried in soil of a peach orchard in Davis, CA, in October. **A and B,** 1981–1982. **C and D,** 1982–1983. Circles represent the mean number of colonies of four 10-dilution-dish replicates per three endocarps expressed as the percentage of propagules surviving at time 0; triangles represent the $\log_e$ of these percentages. Y in the equations is the $\log_e$ of the percentage of surviving propagules, and X is the time of sampling in days. Time 0 in the graphs represents 6 March. All correlation coefficients (r) are significant at $P = 0.01$.

fungus that produces abundant chlamydospores (7), which explains its abundant occurrence in dilution dishes kept at room temperature for 2 days.

Isolate CA of *M. piriformis* survived longer than the CH isolate, probably because its sporangiospores withstood higher temperatures than those of the CH isolate (19). In addition, isolate CA, under favorable temperature (21 C) and nutrient availability, sporulated more abundantly than the CH isolate (Table 1, Fig. 3B). These differences could explain differential survival of the isolates on endocarps buried in wet soil. However, because the isolates did not sporulate in dry soil, only their differential sensitivity to high temperatures (19) can explain differences in survival on endocarps buried in dry soil.

Propagule survival of *M. piriformis* on endocarps in the field showed a similar trend to that of propagules mixed with and buried in soil in two peach orchards (17). In addition, isolate CA survived longer than isolate CH, as indicated by the last sampling.

Previous studies showed that sporangiospores of *M. piriformis* can germinate and the fungus can produce a few sporangia in soil amended with plant material or with low concentrations of glucose (18). Presence of similar carbon sources derived from the endocarps favored the germination of sporangiospores and the direct development of sporangia without extensive mycelium. Isolate CA on WA showed <1% germination with direct production of a single sporangium, whereas, on EWA, approximately 50% of its sporangiospores germinated, usually producing one sympodially branched sporangiophore with two to three sporangia.

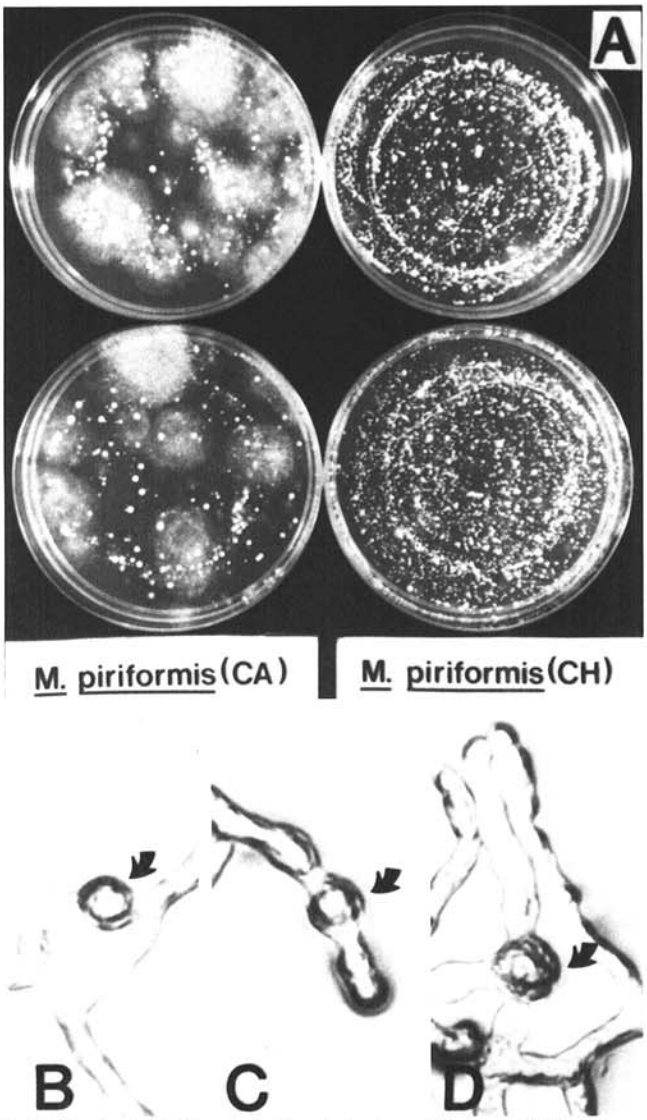

Fig. 7. Survival of *Mucor piriformis* (isolates CA from California and CH from Chile) artificially inoculated on peach and nectarine endocarps after 1 yr in the field. **A,** Dilution dishes from endocarp washings after 2–3 days incubation at 21 C. Isolate CA survived and produced colonies (large diffuse colonies), whereas CH did not. Most of the small colonies are *Cladosporium herbarum*. **B–D,** Colonies of *M. piriformis* originating from sporangiospores (arrows) were observed in dilution dishes (×130). Sporangiospores have one **(B)**, two **(C)**, or three **(D)** germ tubes.

Some pathogens have life cycles that normally include an overwintering stage in or on the soil. Grove et al (10) showed that mummified strawberries infected by the leather rot fungus (*Phytophthora cactorum* (Lebert & Cohn) Schröter) were overwintering sites of its oospores. Mummified peach and nectarine fruits in California orchards serve as overwintering sites for the brown rot fungus *Monilinia fructicola* (Winter) Honey (26). When peaches and nectarines are infected by *M. piriformis*, they may become mummified under dry conditions. *M. piriformis* can overwinter as sporangiospores or chlamydosporelike structures on mummified peaches and nectarines, but, as fruit tissues disintegrate later in the season (spring and summer), the fungus could survive as sporangiospores. All colonies not covered by soil particles in the dilution dishes were initiated from germinated sporangiospores. Under favorable environmental conditions, sporangiospores produced from growth and sporulation of the fungus in the ridges and/or in the protected cavity of endocarps increase soil populations and may act as an additional source of inoculum for the following season.

These results show that peach and nectarine endocarps could be important sites for survival of *M. piriformis* during both winter and summer because endocarps remain in orchards much longer than other mesocarp tissues infected by the fungus, nutrients or small pieces of fruit mesocarp tissue adhering to the endocarp can support development of the fungus for additional inoculum production, the fungus produces chlamydosporelike structures in aged hyphae that come in contact with soil, sporangiospores contaminating endocarps survive for 1 or more years when soil temperatures remain below 27 C, and the long-term surviving propagules on endocarps are sporangiophores, the number of which decline exponentially over time.

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