Characterization and Comparative Studies of *Mucor* Isolates from Stone Fruits from California and Chile

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ABSTRACT

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Twenty-six isolates of *Mucor* spp. collected from stone fruit, mainly peaches and nectarines, were identified and compared for pathogenicity. Thirteen isolates from California and six from Chile were classified as *M. piriformis*; the other seven included two *M. circinelloides*, three *M. racemosus*, and two *M. plumbeus*. These four species of *Mucor* were also recovered from soil of three stone fruit orchards in Parlier, CA. The isolates of *Mucor* spp. obtained from orchard soil included <1-8% *M. piriformis*; 21-28% *M. racemosus*; 1-5% *M. circinelloides*, *M. plumbeus*, *M. hiemalis*, and *M. genevensis*; and other (59-73%) unidentified *Mucor* spp. Optimum temperature for mycelial growth was 21 C for *M. piriformis* (all isolates), 27 C for *M. racemosus* and *M. plumbeus*, and 30 C for *M. circinelloides*. Maximum temperature for growth of *M. piriformis* was approximately 27 C; 33 C for *M. racemosus* and *M. plumbeus*, and 39 C for *M. circinelloides*. The isolates of *M. piriformis* from peaches and nectarines grew well and sporulated abundantly at 0 C, but the isolate of *M. piriformis* from apricot and *M. racemosus* grew much more slowly, *M. circinelloides* grew very slowly, and *M. plumbeus* did not grow at all after 11 days of incubation. Only the isolates of *M. piriformis* decayed peaches and nectarines at 0 C—the isolates from Chile being the most virulent. At 20 C, however, an isolate of *M. piriformis* from apricot was the most virulent. *M. circinelloides*, *M. racemosus*, and *M. plumbeus* caused decay of Dixon peaches at 20 C. Single-spore inoculations of *M. piriformis* caused an infection of wounded peaches and nectarines. Wounding of peach fruit is necessary for infection by ungerminated sporangiospores of *M. piriformis* but not for infection by germinated sporangiospores of the fungus. Once the fungus is established on fruit, it can spread in storage to surrounding healthy, unwounded fruit during both ripening (20 C) and cold storage (4 C) temperatures.

Additional keywords: Mucorales, postharvest decay

Mucor spp. in general have been considered of minor importance as postharvest pathogens. In a number of instances, however, *Mucor* spp. have caused serious decay of strawberries (*Fragaria* × *ananassa* Duchesne) (8-11), pears (*Pyrus communis* L.) (12,13,20), apples (*Malus domestica* Borkh.) (5,16), peaches (*Prunus persica* (L.) Batsch), nectarines (*P. persica* var. *nectarina* (Aiton) Maxim.) (1,30-32), guava (*Psidium guajava* L.) (18), tomatoes (*Lycopersicon esculentum* Mill.) (3,32), and sweet potatoes (*Ipomoea batatas* (L.) Lam.) (19). The etiology of Mucor decay includes a number of *Mucor* species. The most important species, and those that have been reported previously, are *M. piriformis* A. Fischer (5,11,13,16,20,26), *M. mucedo* P. Mich. ex Saint-Amans (6,9,10,15,16,25), *M. hiemalis* Wehmer (2,18), *M. strictus* Hagem (12), *M. racemosus* Fresen. (5,16,19,21), *M. circinelloides* Tieghem (32), and *M. fragilis* Bainier (13).

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Although Mucor rot of pears and apples can be a serious problem in the Pacific Northwest and southwest Canada in fruit stored for a long period, this rot is generally considered to be of minor importance on stone fruit because it occurs only sporadically. In the United States, for instance, the first report of *Mucor* spp. causing rot on stone fruit in cold storage was made in 1973 by Smith and Lynch (31). In 1977, Anderson et al (1) implicated a species of *Mucor* in the decay of peaches stored in controlled atmosphere. In 1979, Smith et al (32) reported that *M. piriformis* caused decay of peaches and nectarines. In California in 1977, an unusual amount of decay caused by *M. piriformis* developed during transit (at 4-5 C) of fresh-market peaches and fresh-market nectarines from Chile (22). In 1979 and 1980, isolations from different stone fruit in the field and fruit from cold storage revealed that *Mucor* spp. were more common than had been suspected. In 1988 and 1989, Mucor rot caused problems on stored Granny Smith apples, Asian pears, Fuji apples, and Black Diamond plums exported from California to the Orient.

Mucor spp. belong to the class of Zygomycetes and occur typically as saprophytes on soil and dung. Two species, however (*M. mucedo* and *M. piriformis*), are the most prevalent fungi that cause decay on strawberries and pome fruit. Because decay caused by *Mucor* spp. can be easily mistaken for Rhizopus rot, caused by *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. and other *Rhizopus* spp., and because there is no effective control for Mucor decay on either pome or stone fruit, measures aimed at reducing decay must be based on correct diagnosis of the pathogen and a better understanding of the pathogen's behavior. To date, only limited information has been published on the characterization of *Mucor* spp. from peaches and nectarines in the western United States (22). Therefore, this study was initiated with the following objectives: 1) to characterize the isolates of *Mucor* spp. recovered from stone fruits in California and fruit imported from Chile; 2) to compare their pathogenicity on peaches, nectarines, and other stone fruits; and 3) to understand the ways the fungus infects stone fruit and how decay spreads in storage in order to help develop control methods.

MATERIALS AND METHODS

Collection of *Mucor* spp. from decayed stone fruit. Isolations of *Mucor* spp. from decayed fruit in the field or from samples sent to the laboratory were made on acidified potato-dextrose agar (APDA) slants and dishes, respectively. In addition, isolations from samples of decayed nectarines imported from Chile were made on APDA. All isolates were single-spored and stored in test tubes containing 5% (w/w) wheat bran mixed with autoclaved soil. This medium extends survival and minimizes fungal character shifting by infrequent subculturing of the isolates. Keys for the Mucorales (14,28,29,35) were used to identify the recovered *Mucor* spp. by microscopic examination. In addition, the identified isolates of *M. piriformis* were crossed with ATCC 58344 (+) and ATCC 58343 (−) of *M. piriformis* (isolated from pear fruit) to determine their mating type (24). Three representative isolates were deposited with ATCC (one each from a decayed peach, isolate CA = ATCC 52555; from a decayed nectarine, isolate CH = ATCC 52554; and one from a decayed apricot, isolate AP = ATCC 52553).

Soil sampling and assay procedures. One quantitative and two qualitative methods were used to determine *M. piriformis* or *Mucor* spp. in soil from three representative stone fruit orchards in the San Joaquin Valley. Five soil samples, each consisting of five composite soil cores taken from a soil depth of 0–5 cm with a soil tube sampler (2 cm i.d.), were collected from one nectarine and two peach orchards in Parlier, CA (center of the fresh-market peach and nectarine growing area), in September 1981. Soil samples were assayed three different ways: 1) 3 g of soil was added to 150 ml of potato-dextrose broth (PDB) amended with 100 μl of novobiocin per milliliter of medium (4), which was then shaken for 2 hr every 10 days until the first mycelial colonies developed on the surface of the medium, and finally incubated for two additional months at 0 C; 2) peach (cv. Halloween) and plum (*Prunus domestica* L. 'Casselman') fruit were wounded with a glass rod (2 mm diameter), 0.25 g of orchard soil was placed in each wound, and the fruit were incubated at 0 C for 1 mo; and 3) a standard dilution plate technique was used in which 100 μl of each soil dilution was spread onto five APDA dishes (23). This experiment was repeated in March 1989 and results between the two experiments were averaged.

Morphological characteristics. For identification purposes, the three isolates of *M. piriformis* (CA, CH, and AP) (Table 1) and one each of *M. circinelloides*, *M. racemosus*, and *M. plumbeus* Bonord. were cultured on synthetic Mucor agar (SMA) (17) for 5 days at 21 C. The SMA contained 40 g of dextrose, 0.5 g of KH_2PO_4, 2 g of L-asparagine, 0.25 g of $MgSO_4$, 100 μg of thiamine chloride, and 15 g of agar in 1 L of distilled water. The pH after autoclaving the medium was 4.8. The dimensions of sporangia, columellae, sporangiophores, and sporangiospores were determined per species. In addition, the height of the mycelium and sporangiophores (turf) for *M. piriformis* was measured in test tubes (15 × 2 cm) containing malt extract agar (MEA) or SMA after 10 days of incubation in darkness at 21 C. The isolates of *M. piriformis* and *M. circinelloides* from California were compared with *M. piriformis* (an isolate from peaches) and *M. circinelloides* (an isolate from tomato) obtained from Beltsville, MD (courtesy of H. E. Moline). The isolates of *M. racemosus* and *M. plumbeus* collected from stone fruits were compared with known isolates of these species (courtesy of E. E. Butler, University of California, Davis).

Temperature/growth studies. A 5-mm mycelial plug cut from the margin of vigorously growing 3-day-old cultures of the isolates was transferred in the center of petri dishes containing SMA and the dishes were incubated in darkness at 0–39 C at three-degree intervals. The SMA dishes were equilibrated at the respective temperatures for 24 hr before placement of the mycelial plug. Radial growth of colonies was recorded daily and expressed as millimeters of growth within a 24-hr period. In another experiment, SMA dishes were equilibrated at 0 C for 24 hr; a 5-mm mycelial plug from each *Mucor* sp. was transferred to the center of each dish and the dishes were incubated at 0 C for 10–11 days. Radial growth of *Mucor* spp. was recorded daily.

Pathogenicity and host specificity studies. Pathogenicity of the four *Mucor* spp. was tested on Dixon peaches, Autumn Grand nectarines, Casselman plums, Bing cherries (*P. avium* (L.) L.), and Royal apricots (*P. armeniaca* L.) harvested from experimental orchards of the University of California. After harvesting, fruits were surface-disinfested with 0.084% NaOCl for 3 min, allowed to dry, placed in a plastic container over waxed wire-mesh (eight fruits for peaches and nectarines and 10 fruits for cherries and apricots per container), and inoculated by making a puncture wound (3 mm in diameter) with a sharp glass rod and placing 50 μl of a suspension of 3 × 10^4 spores per milliliter in each wound. Inoculated fruits were incubated at either 0 or 20 C. Fruits held at 0 C were equilibrated at this temperature for 24 hr after surface disinfestation and then inoculated. The percentage of infected fruit and the diameter of decay lesions were measured 2 and 4 days after inoculation on fruit kept at 20 C and 10 and 20 days on fruit held at 0 C. In addition, the amount of decayed tissue was determined by weighing the fruits before inoculation and after removing decayed tissues on the last day of incubation for fruit held at 0 C and after 6 days at 20 C. To determine any fruit weight losses during the incubation period, uninoculated (control) fruit was weighed at the beginning and the end of each incubation period at 0 and 20 C, respectively.

Inoculations of stone fruits with single spores of *M. piriformis*. To determine whether single spores of *M. piriformis* are able to infect and cause decay, peaches (cv. Loadel) were surface-disinfested and wounded as described previously and inoculated with a single spore of *M. piriformis*. Single spores were obtained from water-agar dishes spread with 100 μl of a suspension of 20,000 spores per milliliter of either a CA or CH isolate of *M. piriformis*. In another experiment, germinated single spores were obtained from APDA dishes and placed in wounded Loadel peaches. Inoculated peaches were placed in four plastic containers (six fruits per container) and incubated at 20 C for 4 days, after which the percentage of infected peaches and the average diameter of lesions were determined by measuring a vertical and a horizontal diameter of each lesion.

Effect of fruit firmness on decay. To determine the effect of fruit tissue firmness on decay, 16 Dixon peaches (four replicates of four fruit each) were inoculated as described previously with a suspension of 3 × 10^5 sporangiospores per milliliter of each CA, CH, and AP isolate of *M. piriformis*, *M. circinelloides*, and *M. racemosus* on the stylar end of each fruit and incubated at 20 C for 4 days. For each decay lesion, two vertical diameters were measured once, along and opposite the fruit suture, and again along the left and right cheeks. The tissue firmness in the suture, opposite the suture and on the left and right cheeks of 10 peaches was determined with a Hunter spring force gage (AMETEK, Testing Equipment Systems, Lansdale, PA) with a compression head 8 mm in diameter. The content of soluble solids (sugars) on each of the four sites of 10 peaches was determined with a hand refractometer (ATAGO, NSG Precision Cells, Inc., Hicksville, NY).

Effect of wounding in infection of stone fruits. Fay Elberta peaches were harvested from an experimental orchard at the University of California Kearney Agricultural Center and surface-disinfested as described previously. The peaches were inoculated with spore inocula obtained in the following manner from a culture of the fungus grown in dishes containing APDA. Sporangiospores taken from the cover of a petri dish and

Table 1. Frequency of isolation of *Mucor* spp. from various stone fruits in California

Mucor spp.	Isolates (no.)	Source/origin	Year of collection	Mating type
M. circinelloides	1	Peach/Modesto	1962	ND[a]
	1	Cherry/Stockton	1980	ND
M. piriformis (isolate AP)	1	Apricot/Winters	1962	+
M. piriformis (isolate CA)	10	Peach/Parlier	1977	[1]−, [1]+, [8] Neutral
M. piriformis (isolate CH)	6	Nectarine/Chile[b]	1977	+
M. piriformis	2	Plum/Fresno	1978,1989	Neutral
M. plumbeus	2	Apricot/Winters	1980	ND
M. racemosus	1	Prune/San Jose	1973	ND
	1	Apricot/Brentwood	1980	ND
	1	Nectarine/Fresno	1980	ND

[a] Not determined.
[b] Fruit imported to California from Chile.

adjusted to 1×10^6 per milliliter were plated on APDA dishes and incubated at 20 C for 8–12 hr to allow for 98–100% spore germination. The germinated sporangiospores were removed by adding 4 ml of water in each petri dish and adjusted to 3×10^3 germinated spores per milliliter (unwashed/germinated treatment). Other sporangiospore suspensions prepared in the same way were washed three times with sterile distilled water by centrifuging them for 2 min at 5,000 *g* to remove culture nutrients (washed/germinated treatment). A third sporangiospore suspension was prepared by washing sporangiospores deposited on the covers of petri dishes of a 3-day-old culture with distilled water and adjusting to 3×10^3 sporangiospores per milliliter in a 1:1 distilled water:peach juice (7.5° Brix) mixture obtained by aseptically squeezing 10 Fay Elberta peaches (ungerminated/peach juice treatment). Four peaches each in three plastic containers were inoculated without wounding by placing 100 μl of each type of sporangiospore suspension in 0.5-cm-long plastic tubes cut from a tygon tube (6 mm i.d.) and attached to the fruit with high-vacuum petroleum jelly. Unwounded peaches inoculated with washed ungerminated sporangiospores served as controls (washed/ungerminated treatment). The experiment was repeated twice.

Secondary spread of *M. piriformis* in storage. To determine whether *M. piriformis* will spread from infected to healthy fruit in storage, Fay Elberta peaches were surface-disinfested in 0.084% chlorine solution and wound-inoculated with a suspension of 2×10^4 sporangiospores per milliliter of each CA or CH isolate of *M. piriformis*. Four containers with three peaches each were incubated at 20 C for 12 days or at 4 C for 25 days. Four days later, three unwounded healthy peaches were placed carefully at the side of each inoculated fruit so that they touched each other. Healthy peaches were examined for infection 1, 2, 5, and 8 days after placement by the infected fruit at 20 C and 25 days for those incubated at 4 C. The experiment was repeated twice. Unless otherwise indicated, all experiments were repeated at least once.

Statistical analysis. Data were analyzed by ANOVA, and when *F* values were significant, means were compared with Duncan's multiple range test for mean differences with Statistical Analysis Systems (SAS) software (SAS Institute Inc., Cary, NC).

RESULTS

Collection of *Mucor* spp. from decayed stone fruit. Four distinct *Mucor* spp. (*M. piriformis*, *M. circinelloides*, *M. racemosus*, and *M. plumbeus*) were isolated from stone fruits in California (Table 1). Most of the isolates causing decay of fruits were *M. piriformis*, followed by *M. racemosus*. Determination of the mating type of the isolates of *M. piriformis* indicated that there are three distinct types in nature: +, −, and those that did not mate with the tester mating types or with each other (neutral) (Table 1).

Soil sampling and assay procedures. All three assay procedures were effective in isolation of *M. piriformis* and *M. racemosus* when flasks, fruit inoculated with soil, and dilution dishes were incubated at 0 C. The soil from the three stone fruit orchards contained 12.8–19.3 $\times 10^4$ propagules of *Mucor* spp. per gram of dry soil (Table 2). Of these, <1–8% were *M. piriformis*, 21–28% were *M. racemosus*, and 64–78% were other *Mucor* spp., including *M. circinelloides*, *M. hiemalis*, *M. genevensis* Lendner, and *M. plumbeus*, which developed after incubation of the dilution dishes at 23 ± 1 C for two more days.

Morphological characteristics. Although there were small differences among isolates of the same species, there were noticeable differences in both macroscopic and microscopic features among the different species (Table 3). The differences between the two groups of isolates of *M. piriformis* from California and Chile were minor, with the exception

Table 2. Propagules of *Mucor* spp. recovered from soil of three stone fruit orchards in Parlier, CA, in 1981 and 1989

Type of orchard	Total propagules of *Mucor* spp. ($\times 10^4$) per gram of dry soil	*Mucor* spp.	Percent
Nectarine (commercial orchard)	12.8 a[x]	*Mucor* spp.	78[y]
		M. racemosus	22
		M. piriformis	<1
Peach (commercial orchard)	16.2 a	*Mucor* spp.	64
		M. racemosus	28
		M. piriformis	8
Peach (UCKAC[z] experimental orchard)	19.3 a	*Mucor* spp.	78
		M. racemosus	21
		M. piriformis	<1

[x] Numbers followed with the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).
[y] *Mucor* spp. include *M. circinelloides*, *M. plumbeus*, *M. hiemalis*, *M. genevensis* (all together 1–5%), and other unidentified *Mucor* spp.
[z] UCKAC = University of California Kearney Agricultural Center.

Table 3. Morphological measurements of *Mucor* spp. grown on synthetic Mucor agar

Mucor spp.	Kind of measurement	Morphological structure measured in μm: Sporangium[a]	Columella[a]	Sporangiophore diameter[a]	Sporangiospores[b]: From tall sporangiophores	Sporangiospores[b]: From short sporangiophores
M. circinelloides	Range	96–122	24–48 × 24–42	9–19	3.1–9.2 × 3.1–4.6	
	Average	104	34 × 33	13	5.7 × 4.1	
M. circinelloides	Range	72–125	23–44	6–12	4.5–7.6 × 3.1–6.1	
(Beltsville)	Average	95	34	10	6.1 × 4.0	
M. piriformis	Range	149–346	126–192 × 96–150	24–42	4.6–16.8 × 4.6–10.7	
(isolate AP)[c]	Average	236	146 × 112	33	10 × 8	
M. piriformis	Range	122–283	86–187 × 67–144	24–48	4.6–9.2 × 3.1–6.1	6.9–12.2 × 6.1–10
(isolate CA)[c]	Average	154	128 × 97	33	7.3 × 4.6	9.2 × 8.1
M. piriformis	Range	154–264	86–182 × 58–144	29–48	6.1–9.2 × 4.6–6.1	6.9–11.5 × 6.1–9.2
(isolate CH)[c]	Average	207	130 × 103	40	7.8 × 5.3	8.6 × 7.8
M. piriformis	Range	120–259	77–182 × 62–154	24–54	3.1–9.2 × 3.1–4.6	
(Beltsville)	Average	162	125 × 101	38	5.7 × 4.1	8.7 × 6.5
M. racemosus	Range	76–96	19–41 × 17–35	8–17	4.6–7.6 × 4.6–7.6	
	Average	75	35 × 30	11	6.7 × 5.8	

[a] Average values for sporangium, columella, and sporangiophore diameter are from 40 measurements.
[b] Average values for spore dimensions are from 100 sporangiospores.
[c] AP isolate of *M. piriformis* is from apricots, CA is from peaches, and CH is from nectarines.

of the height of their mycelium and sporangiophores; the isolate CA from California produced higher turf on all media used (Fig. 1). In this characteristic, the isolate of *M. piriformis* from Beltsville resembled the isolate CH from Chile (Fig. 1). Microscopically, the isolates of *M. piriformis* from peaches (CA) and from nectarines (CH) did not differ greatly from the isolate of *M. piriformis* from peaches from Beltsville, MD. Similarly, *M. circinelloides* had characteristics very similar to the isolate from Beltsville. *M. piriformis* from apricots (AP), however, had very distinct characteristics—the average size of its sporangia, columellae, diameter of sporangiophores, and sporangiospores were larger than those of either CA or CH isolates (Table 3).

Temperature/growth studies. Optimum temperature for mycelial growth was 21 C for *M. piriformis* (both isolates CA and CH), 21–24 C for *M. piriformis* (AP), 27 C for *M. racemosus* and *M. plumbeus*, and 30 C for *M. circinelloides* (Fig. 2). Maximum temperature for growth of *M. piriformis* was approximately 27 C; for *M. racemosus* and *M. plumbeus* was 33 C; and for *M. circinelloides* was 39 C (Fig. 2). The isolates CA and CH of *M. piriformis* and that from Beltsville grew relatively well at 0 C, but the isolate of *M. piriformis* from apricots and isolates of *M. racemosus* grew much more slowly (Fig. 3). Both isolates of *M. circinelloides* showed very slow growth at 0 C and *M. plumbeus* did not grow at all after 11 days of incubation at 0 C (Fig. 3).

Pathogenicity and host specificity tests. All three *M. piriformis* isolates tested caused decay of peaches and nectarines at 0 (Fig. 4 and Table 4) and at 20 C (Table 5). At 0 C, the isolate CH (Fig. 4A) caused significantly ($P < 0.05$) larger lesions and greater amounts of decayed tissue on both peaches and nectarines than the other two isolates (Fig. 4B,C and Table 4). The decay on peaches and nectarines incubated at 0 C became measurable 7–8 days after inoculation with isolate CH and 10–12 days after inoculation with isolate CA of *M. piriformis*. Sporangiophores and sporangia appeared on the 15th day after inoculation, initially in the inoculated wounds, and by the 20th day, all lesions were covered with mature pale olive gray to pale gull gray (27) (gray to black) sporangia. *M. circinelloides*, *M. racemosus*, and *M. plumbeus* did not cause any decay of peaches and nectarines after 20 days of incubation at 0 C (Table 4).

At 20 C, the isolate of *M. piriformis* from apricot caused significantly larger lesions on nectarines but not on peaches after 4 days and significantly greater weight of decayed tissue than that caused by the two other isolates of *M. piriformis*

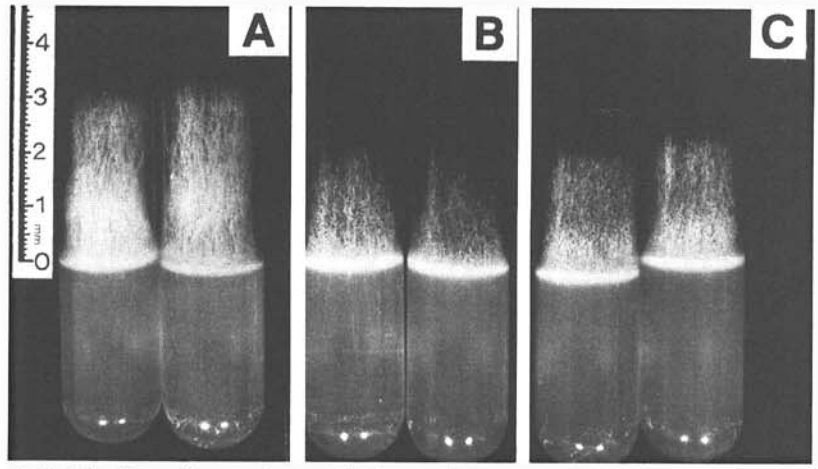

Fig. 1. Height of mycelium and sporangiophores of *Mucor piriformis* grown on malt extract agar at 21 C after 10 days in darkness. **(A)** Isolate CA from California, **(B)** isolate CH from Chile, **(C)** and isolate of *M. piriformis* from Beltsville, MD.

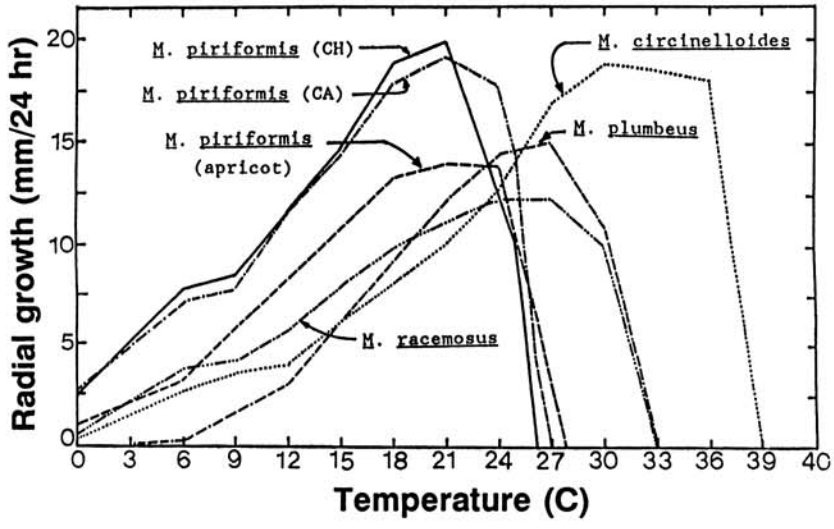

Fig. 2. Effect of temperature on radial growth of *Mucor* spp. on synthetic Mucor agar (SMA) in vitro. (*M. piriformis* [apricot] = isolate AP)

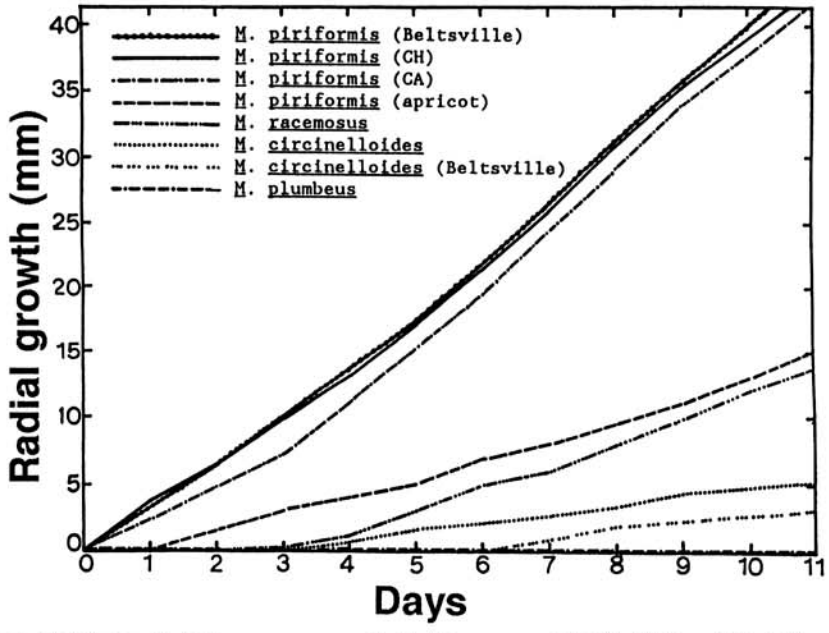

Fig. 3. Radial growth of *Mucor* spp. on synthetic Mucor agar at 0 C for 11 days. (*M. piriformis* [apricot] = isolate AP)

on both peaches and nectarines (Table 5). The saturated atmosphere inside the plastic containers prevented any fruit weight losses of uninoculated (control) fruit. Decay was first noticeable 24 hr after inoculation with *M. piriformis*, and by the second day, young sporangiophores appeared on the tan, water-soaked, soft lesions. *M. circinelloides*, *M. racemosus*, and *M. plumbeus* caused decay only on peaches, and the diameter of lesions was significantly smaller than that of lesions caused by *M. piriformis*. Although these *Mucor* spp. did not cause measurable decay on nectarines (Table 5), they did develop sporangiophores and mature sporangia characteristic of each species but only in the inoculated wound.

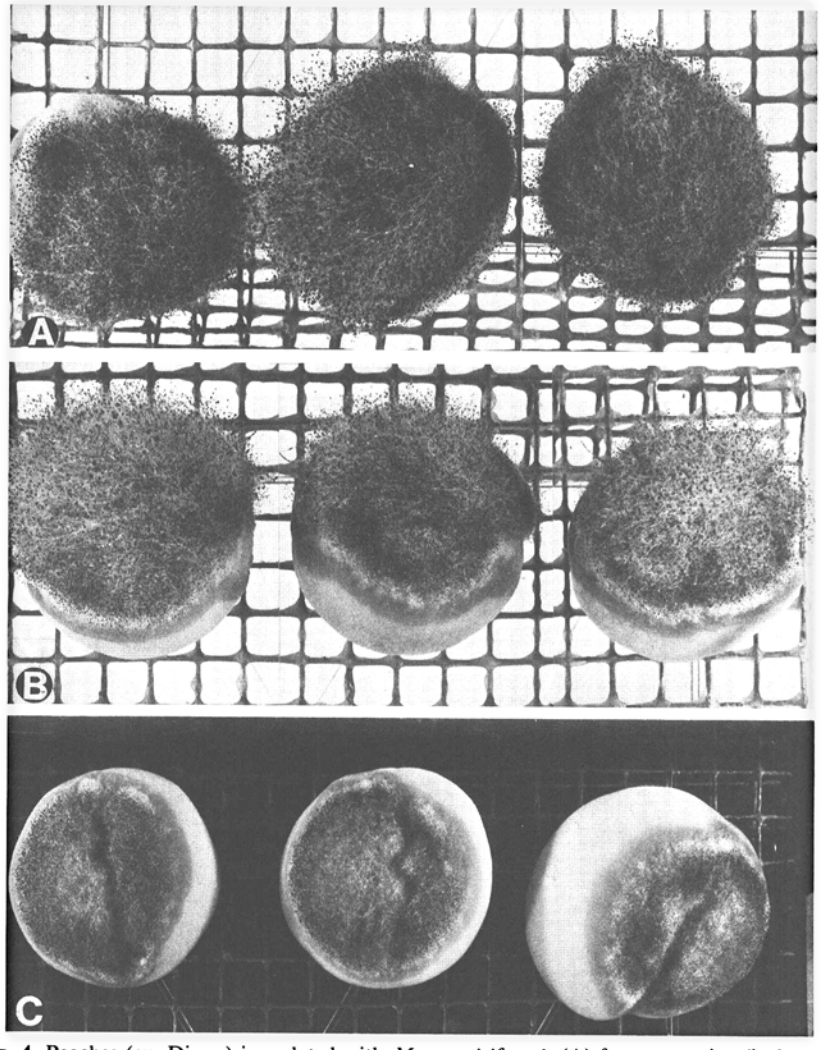

Fig. 4. Peaches (cv. Dixon) inoculated with *Mucor piriformis* **(A)** from nectarine (isolate CH from Chile), **(B)** from peach (isolate CA from California), and **(C)** from apricot (isolate AP from California) and incubated at 0 C for 20 days.

Of the 26 *Mucor* isolates studied (19 *M. piriformis*, two *M. circinelloides*, three *M. racemosus*, and two *M. plumbeus*), only the isolates of *M. piriformis* were pathogenic to both cherries and apricots at 20 C (Table 6); however, *M. plumbeus* was pathogenic to apricots and produced lesions of 6 mm 4 days after inoculation.

Inoculations with single spores of *M. piriformis*. After 4 days of incubation at 20 C, 96% of the peaches inoculated with single spores of isolate CA of *M. piriformis* decayed, with an average lesion diameter of 40 mm. Seventy-five percent of those inoculated with single spores of isolate CH decayed, with an average lesion diameter of 44 mm. The uninfected fruits indicated that either the inoculations were not successful or the single spores transferred with the small water agar piece failed to germinate. However, when a single germinated sporangiospore of *M. piriformis* was placed in wounded peaches, these inoculations resulted in 100% infection for both isolates CA and CH.

Effect of fruit firmness on decay development. Decay lesions that developed at the stylar end on peaches 4 days after incubation at 20 C were ellipsoidal, i.e., the diameter connecting the suture to the opposite of the suture side was significantly larger (56–64 mm) than the diameter (52–58 mm) connecting the right and the left cheek of the fruit. This was true for the three isolates of *M. piriformis* and for *M. racemosus* but not for *M. circinelloides*. The tissue firmness on the suture and opposite to the suture side ranged from 2.6 to 2.8 kg/cm^2, while that on the right and left cheeks ranged from 4.0 to 4.1 kg/cm^2. The content of soluble solids (sugars) in all four sites ranged from 8.9 to 9.5%.

Effects of wounding in infection of stone fruits. Preliminary experiments indicated that wounding is necessary for efficient infection of peaches and nectarines from *M. piriformis*. Only very ripe fruit (with firmness pressure < 4.0 kg/cm^2) can be infected by ungerminated spores of *M. piriformis* without a wound

Table 4. Decay of Dixon peaches and Autumn Grand nectarines inoculated with *Mucor* spp. and stored at 0 C

	Peaches[v]		Nectarines[w]		Percent weight of decayed fruit tissue[x]	
	Mean lesion diameter (mm) after		Mean lesion diameter (mm) after			
Mucor spp.	10 days	20 days	10 days	20 days	Peaches	Nectarines
M. piriformis (isolate CH)[y]	11 a[z]	46 a	7 a	36 a	84 a	66 a
M. piriformis (isolate CA)	4 b	32 b	0 c	21 b	51 b	22 b
M. piriformis (isolate AP)	4 b	25 b	3 b	24 b	21 c	17 b
M. circinelloides	0 c	0 c	0 c	0 c	0 d	0 c
M. racemosus	0 c	0 c	0 c	0 c	0 d	0 c
M. plumbeus	0 c	0 c	0 c	0 c	0 d	0 c

[v] Harvested 21 July.
[w] Harvested 28 July.
[x] Determined 28–30 days after incubation.
[y] Isolate CH is from decayed nectarine (Chile), isolate CA is from decayed peach (California), and isolate AP is from decayed apricot (California).
[z] Means in columns followed by a common letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

Table 5. Decay of Dixon peaches and Autumn Grand nectarines inoculated with *Mucor* spp. and stored at 20 C

Mucor spp.	Peaches[u] Mean lesion diameter (mm) after 2 days	Peaches[u] Mean lesion diameter (mm) after 4 days	Nectarines[v] Mean lesion diameter (mm) after 2 days	Nectarines[v] Mean lesion diameter (mm) after 4 days	Percent weight of decayed fruit tissue[w] Peaches	Percent weight of decayed fruit tissue[w] Nectarines
M. piriformis (isolate CH)[x]	24 a[y]	55 a	10 b	34 b	70 b	48 b
M. piriformis (isolate CA)	20 a	54 a	11 b	34 b	67 b	39 c
M. piriformis (isolate AP)	25 a	57 a	17 a	45 a	81 a	91 a
M. circinelloides	6 c	18 c	0 c	0 c	ND[z]	0 d
M. racemosus	10 b	30 b	0 c	0 c	ND	0 d
M. plumbeus	10 b	30 b	0 c	0 c	ND	0 d

[u] Harvested 21 July.
[v] Harvested 28 July.
[w] Determined 6 days after inoculation.
[x] Isolate CH is from decayed nectarine (Chile), isolate CA is from decayed peach (California), and isolate AP is from decayed apricot (California).
[y] Means in columns followed by a common letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).
[z] ND = not determined because of frequent contamination with *Rhizopus stolonifer*.

Table 6. Decay of Bing cherries and Royal apricots at 20 C, 2 days after inoculation with *Mucor* spp.

Mucor spp.	Mean lesion diameter (mm) Cherries	Mean lesion diameter (mm) Apricots
M. piriformis (isolate CH)	13 a[z]	23 a
M. piriformis (isolate CA)	15 a	20 a
M. piriformis (isolate AP)	15 a	20 a
M. circinelloides	0 b	0 b
M. racemosus	0 b	0 b
M. plumbeus	...	0 b

[z] Means in columns followed by a common letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

Table 7. Effect of inoculum condition of *Mucor piriformis* on infection of unwounded Fay Elberta peaches[w] incubated at 20 C for 3 days

Isolate of *M. piriformis*	Condition of spore inoculum	Mean diameter of decay lesion (mm)[x]	Infected fruit (%)
CA			
	Unwashed/germinated[y]	23 a[z]	100 a
	Washed/germinated[y]	5 b	25 b
	Ungerminated/peach juice	29 a	92 a
	Washed/ungerminated (control)	0 b	0 b
CH			
	Unwashed/germinated[y]	22 b	100 a
	Washed/germinated[y]	5 c	25 b
	Ungerminated/peach juice	31 a	100 a
	Washed/ungerminated (control)	1 c	8 c

[w] Peaches had 4 kg/cm^2 firmness, 7.8% soluble solids, and pH 3.8.
[x] Values are the average from a test with three four-fruit replications.
[y] Spores were allowed to germinate on APDA dishes incubated at 21 C for 8–12 hr.
[z] Numbers in each column for each isolate followed by the same letter are not significantly different according to Duncan's multiple range test ($P < 0.05$).

(T. J. Michailides, *unpublished*). Unwashed, germinated spores of both isolates (CA and CH) resulted in 100% infection of inoculated peaches, whereas only 25% of the fruit was infected when washed germinated spores were used as inocula (Table 7). Ungerminated sporangiospores provided with nutrients (peach juice) infected 92–100% of the peaches 3 days after inoculation causing decay lesions of 29–31 mm in diameter. Washed ungerminated spores of isolate CA did not infect any peaches 3 days after inoculation, whereas those of isolate CH infected 8% of the inoculated fruit. Although the differences in percentage of infected fruit ($F = 0.80$, df = 1) and the diameter of lesions ($F = 0.37$, df = 1) were not significant ($P > 0.05$) between the two isolates when germinated spores were used as inocula, the isolate CH was more aggressive than the isolate CA in infecting unwounded peach fruits with washed ungerminated sporangiospores (Table 7).

Secondary spread of *M. piriformis*. At 20 C, 1 day after uninoculated healthy fruit was placed in contact with the decayed fruit, brown discoloration and slippage of the fruit skin could be noticed. Within 2 days, all healthy fruit showed breakdown of the skin. Five days later, hyphae of the fungus colonized the healthy fruit, making it difficult to pull it away from the fruit infected initially. Development of sporangiophores of both isolates was evident on the uninoculated fruit by the eighth day. Secondary spread was found on all experimental fruit incubated at 4 C, with fungal nesting occurring on 81% of the sides after 25 days of incubation. No differences were determined between isolates CA and CH of *M. piriformis*.

DISCUSSION

We showed that among the four species of *Mucor* isolated from stone fruits, only *M. piriformis* could cause significant amounts of decay during cold storage of stone fruits. The reason for its sporadic occurrence is unknown, but it is believed that unsanitary practices during harvest of stone fruit (i.e., picking fruit from the ground) and/or in the packinghouse (32) contribute significantly to Mucor decay in storage. Because registered fungicides are ineffective in controlling *M. piriformis*, Mucor rot of various fruits can be a problem, as it was during the marketing of peaches in 1977 (22), apples in 1988, and plums in 1989.

Ten isolates of *M. piriformis* from peach and two from plum were identical, but the isolate from apricot was morphologically distinct (Table 3). All isolates caused decay of peaches and nectarines at both 0 and 20 C. The six isolates of *M. piriformis* isolated from nectarines shipped from Chile were identical to each other but distinct from the group of California isolates. Morphological differences among isolates of *M. piriformis* of different origin are to be expected. Schipper (28) reported that strains (= isolates) of *M. piriformis* vary in height of turf, diameter of sporangiophores, and the ratio of tall/short sporangiophores. In these studies, the main morphological difference between isolate CA and CH was the height of the turf (Fig. 1). In addition, the isolates from

Chile were + mating type, whereas the majority of the isolates from California were neutral, i.e., did not produce zygospores when mated with either the + or − mating types of *M. piriformis*. Also, the isolates from Chile more closely resembled the isolate of *M. piriformis* obtained from Beltsville, MD (Fig. 1). The isolate from apricot resembled *M. wosnessenskii* Schostakowitsch, which is considered a synonym of *M. piriformis* (28). This isolate had a slower growth rate than the other isolates, both at 0 and at 3–25 C (Fig. 2). However, isolates of *M. piriformis* from apples and Asian pears collected during 1988–1989 resembled those isolated from plums in 1978 and 1989 (T. J. Michailides, *unpublished*).

M. piriformis has been reported as the cause of postharvest decay of peaches and nectarines (1,32). Most of the isolates obtained in this study were from freestone peaches and nectarines and the remainder from plums, apricots, and cherries. Sources for the other species (*M. circinelloides*, *M. racemosus*, and *M. plumbeus*) were peach, prune, and apricot (Table 1). All these *Mucor* spp. were also recovered from soil samples collected from stone fruit orchards in California. With the exception of one report (22), none of these *Mucor* spp. has been previously reported to cause postharvest decay of stone fruit in California, although *M. racemosus* has been reported as causing decay of sweet potatoes stored at 0–5 C for several weeks (15). In this study, *M. racemosus* showed some growth at 0 C after 10–11 days of incubation (Fig. 3).

Peaches and nectarines shipped from California to the eastern states are usually held in transit and storage for about 20 days before being placed on the market. It is assumed that stone fruits shipped from Chile to the United States would be expected to last for the international and the domestic shipping and handling periods that logically exceed 20 days. To extend their market life, stone fruits, as well as other fruits and vegetables, are stored at temperatures near 0 C. Storage at such low temperatures inhibits development of many postharvest pathogens. We found, however, that *M. piriformis* isolates grew and infected peaches and nectarines at 0 C and caused considerable decay within a relatively short time. The ability of *M. piriformis* to cause decay at 0 C results, at least in part, from the fact that its sporangiospores lose neither viability nor the ability to germinate and grow at this temperature (7).

All four species, *M. piriformis*, *M. circinelloides*, *M. racemosus*, and *M. plumbeus*, caused serious postharvest decay in peaches incubated at 20 C, a temperature at which peaches are usually held for ripening, but only *M. piriformis* caused decay of stone fruits at 0 C. Therefore, *M. circinelloides*, *M. racemosus*, and *M. plumbeus* should not be considered as postharvest pathogens during cold storage. Although peaches and nectarines are close relatives botanically, *M. circinelloides*, *M. racemosus*, and *M. plumbeus* did not infect nectarines. We did not investigate this difference, but it could well be the subject of another study to determine why nectarines were immune to infections by these species while peaches were not. The differences among isolates in lesion size and amount of decayed tissues at 0 C can be explained only in part by differences in growth rates. Isolate CH, which showed better growth rates at 0 C than the other isolates of *M. piriformis*, caused larger lesions. In contrast, the isolate of *M. piriformis* from apricots, which grew the least at 0 C of the *M. piriformis* isolates, caused the smallest diameter decay lesions on peaches incubated at 0 C for 20 days (Fig. 4). At 20 C, however, the isolate from apricots caused larger decay lesions than the other isolates, although its mycelial growth rate was again the lowest of the *M. piriformis* isolates. Therefore, other factors, such as enzyme production, may play a more significant role in lesion development than growth rate alone.

In general, wounding was necessary for infection of stone fruit by *M. piriformis*. Similarly, wounding is necessary for infection of guava fruit by *M. hiemalis* (18). It seems that nutrients diffusing from the wound help spore germination and, subsequently, infection. Germinated sporangiospores, however, were able to infect unwounded tissues. Significantly higher percentages of fruit were infected with unwashed sporangiospores than with washed (Table 7), indicating that the presence of nutrients and/or enzymes in the spore suspension is critical for the infection of unwounded fruit. When suspensions of ungerminated sporangiospores were provided with nutrients (peach juice), they caused infection of unwounded fruit (Table 7).

Smith et al (32) reported the relationship between maturity of fruit infected with *M. piriformis* and the development of decay on peaches and tomatoes. Spotts (33) found that susceptibility to decay caused by *M. piriformis* increased with maturity in pear (Anjou and Bartlett) fruits. Findings of this study agree with these results. Significant differences in tissue firmness in various areas of the fruit resulted in differences in the rate of decay so that initially round lesions become ellipsoidal. Further studies are needed to determine whether the fungus mycelium has difficulty in penetrating fruit with greater firmness or whether the fungus lacks enzymes necessary to dissolve the immature fruit tissue. In addition, significant differences in the content of soluble solids may also play an important role in the varying rates of decay.

Because the sporangiospores of *M. piriformis* supplied with peach juice can infect unwounded peaches, secondary spread in storage from dripping juice is expected, as was shown in a pear study (2). In addition, the fungus can infect and colonize healthy fruit by moving from an infected fruit in a way similar to that of *R. stolonifer* (34). Sporangia touching healthy fruit tissue dissolve the unwounded skin and colonize the healthy fruit. The use of plastic Panta-pak fruit holders in boxes helps prevent the secondary spread, although cracks on the bottom may allow the juice to drip and contaminate healthy fruit in lower layers of boxes. When cost permits, pears are wrapped individually in paper impregnated with copper, which minimizes secondary spread of Mucor decay (2), but this is not practical for stone fruits at the present time.

In summary, these results indicate that among four species of *Mucor* isolated from stone fruits and soils from stone fruit orchards in California, only *M. piriformis* has the potential to cause significant losses in stone fruit industries. Fortunately, Mucor rot is not a problem every year. However, when it occurs, because the fungicides registered for stone fruits are ineffective against Mucor rot, the fungus can destroy large quantities of fruits very quickly in cold storage. In addition, unsanitary harvest conditions (i.e., contamination of harvest bins with orchard soil containing propagules of *M. piriformis*) may be a way of contaminating fruit, as was shown to be the case for Mucor decay in pear orchards in Hood River, OR (23).

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