

Studies with *Mucor* Species Causing Postharvest Decay of Fresh Produce

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ABSTRACT

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Fifteen isolates of *Mucor piriformis* from peaches or nectarines and five of *M. circinelloides* from tomatoes were studied. Sporangiospores of *M. piriformis* isolates germinated and formed normal germ tubes at temperatures from 0 to 24 C but not at 27 C. At each temperature, the diameter of the spores increased before germination. At 27 C, the spore diameter swelled to six to eight times that of the resting spore. When germination occurred at 27 C, the germ tubes were abnormally swollen and often grotesquely shaped. Exposure to 27 C for 7 or 10 days prevented germination

when the isolates were later transferred to 18 C. Spores of *M. circinelloides* isolates germinated at temperatures up to 40 C and did not swell appreciably. In inoculative studies, both *Mucor* spp. caused decay of stone fruits, apples, pears, oranges, sweet potatoes, tomatoes, carrots, bell peppers, celery, and lettuce but not of white potatoes. Both green and mature peaches and tomatoes decayed. *M. piriformis* was the more pathogenic of the two species. Cultures of *M. piriformis* and *M. circinelloides* have been deposited with the American Type Culture Collection.

Additional key words: host range, host-parasite interaction, maturity studies, storage tests, temperature studies.

In 1973 peaches removed from controlled-atmosphere storage at 0 C were severely decayed by a *Mucor* sp. *Mucor* spp. have been reported to cause decay of numerous fruits and vegetables but

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usually have been considered of minor importance as storage pathogens (3,12,15). Since 1970, however, *Mucor* spp. have been reported to cause serious postharvest decay of strawberries (4-8), pears (9,16), mangoes (13), guava (14), vegetables (17), and peaches (2,20,21). These reports stimulated our studies of *Mucor* spp. as a cause of serious decay of fruits and vegetables.

MATERIALS AND METHODS

Twenty isolates of *Mucor* were collected from fruit-producing areas in Maryland and Virginia. Fifteen isolates of *M. piriformis* Fisher (18) were obtained from decayed peaches or nectarines from commercial packing sheds in central and western Maryland or from wholesale markets. Five isolates of *M. circinelloides* Van Tieghem (19,24) were obtained from decayed tomatoes from a Virginia packing shed or local markets.

Spore germination and mycelial growth studies. Spores, suspended in distilled water, were flooded onto petri plates containing potato-dextrose agar (PDA), or 5-mm mycelial plugs from cultures 24–48 hr old were placed on PDA. All plates were equilibrated at the incubation temperature for 24 hr before inoculation with spores or mycelial plugs. Inoculated plates were held at constant temperatures from 0 to 40 C. We counted the number of germinating spores under the $\times 100$ magnification of a compound microscope and measured spore size at $\times 430$. Germination counts were made hourly and daily; growth measurements from plugs were made daily.

In another study, PDA plates flooded with spores were held for 1, 3, 7, or 10 days at 27 C, then transferred to 18 C. Germination characteristics of the spores were noted when the plates were removed from 27 C and after incubation at 18 C. Plates with mycelial plugs were also placed at 27 C, then transferred to 18 C.

Pathogenicity tests. In the host range study, inoculations were made with needle jab punctures on two sides of stone fruits, apples, pears, tomatoes, oranges, sweet potatoes, white potatoes, carrots, bell peppers, lettuce, and celery. All were held at 18 C after inoculation, and decay was evaluated daily.

The relation of temperature to infection was determined with peaches and tomatoes. In one experiment, mature peaches (10–12 lb, or 44.5–53.4 N, as determined with a Magness-Taylor pressure

recorder) were equilibrated at 0, 4, 10, 13, 20, and 27 C for 24 hr, then inoculated with a representative isolate of *M. piriformis*. Decay was evaluated after 4 and 6 days. In the other test, tomatoes ranging from mature-green to fully red (22) were equilibrated at 10 or 18 C for 24 hr, then inoculated. Decay readings were made on fruit held 3 days at 18 C and 5 days at 10 C.

Pilot storage tests. To test the possibility that *Mucor* spp. infection may be due to unsanitary conditions in packinghouses, we compared decay on handpicked fruit with decay on commercially harvested and treated fruit. Large plastic bags used as storage chambers (2) were placed on pallets in a commercial peach storage house. Each bag contained eight crates of hand-harvested fruit (two from each of four orchards) that had been treated with 100 $\mu\text{g}/\text{ml}$ of benomyl in water at 46 C before storage. An additional 16 crates (four from each orchard) were harvested commercially, handled normally, and cooled in a commercial packinghouse. All fruit were stored 6 wk under one of the following conditions: (i) air storage constantly at 5 C; (ii) air storage at 5 C with 2 days of intermittent warming (air at 18 C every 3 wk, then returned to 5 C); (iii) controlled-atmosphere storage (1% O_2 + 5% CO_2) constantly at 5 C; (iv) controlled-atmosphere storage at 5 C with 2 days of intermittent warming.

RESULTS

Spore germination and mycelial growth. Spores of the *M. piriformis* isolates averaged $6.5 \times 8.5 \mu\text{m}$ but nearly doubled in size and became circular before germination at temperatures below 21 C. At 21 and 24 C they swelled even more; at 27 C they swelled to six to eight times their original size.

Most of the spores held at 18 C germinated within 6 hr and those held at 15 or 24 C germinated within 24 hr. When spores were held

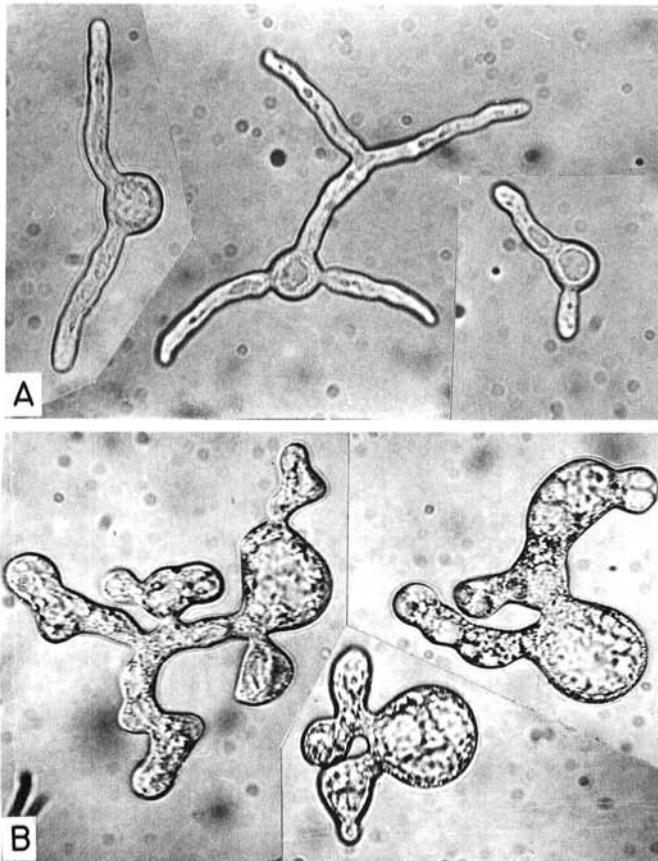


Fig. 1. Germination of *Mucor piriformis* spores. A, Normal swelling and germination of spores at 18 C. B, Abnormal swelling and germination of spores at 27 C. ($\times 430$)

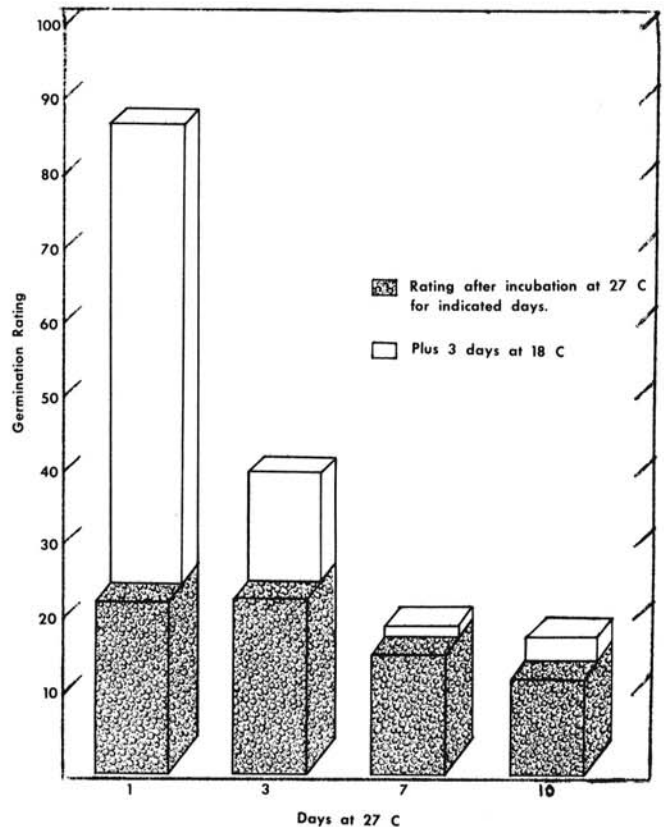


Fig. 2. Germination ratings of *Mucor piriformis* spores held on PDA plates at 27 C for 1, 3, 7, or 10 days, then transferred to 18 C. Many spores held 1 day formed normal germ tubes, but the number decreased considerably for those held 3 days; spores held 7 or 10 days did not form normal germ tubes. Data represent mean ratings of 15 isolates in three tests: 0 = no germination; 10, 15, and 30 = some germination, abnormal germ tubes; above 75 = various degrees of germination, normal germ tubes.

at 4 or 27 C, 60 and 66%, respectively, germinated within 24 hr. Most of the spores held at 0 C germinated within 54 hr.

Germ tubes of spores held at 27 C were abnormal. After 1 day, the spore walls appeared thickened and the germ tubes that did form were greatly swollen and often grotesquely shaped (Fig. 1). Cytoplasm in the spores and germ tubes was usually granular and agglutinated, giving the germinating spore a dark to opaque appearance. Sometimes the spore wall appeared broken and the spores had odd shapes.

In the spores held on PDA plates at 27 C for 1, 3, 7, or 10 days, then transferred to 18 C, abnormal germination, cytoplasmic granulation, and wall breakdown began within 1 day. The longer the spores were held at 27 C, the more severe granulation and wall breakdown became. When spores exposed for 1 day at 27 C were shifted to 18 C, many formed normal germ tubes, and resultant mycelial growth rapidly covered the plates and formed normal sporangiophores (Fig. 2). Nevertheless, many abnormally swollen spores or spores with abnormal germ tubes were seen beneath the normal growth. When the spores were exposed for 3 days at 27 C, the number producing germ tubes was considerably less than after the 1-day exposure (Fig. 2). When germ tubes continued to grow and form colonies at 18 C, sporangiophores were much shorter than normal. Spores held 7 or 10 days at 27 C did not form normal germ tubes.

Spores of the *M. circinelloides* isolates swelled only slightly and formed germ tubes at 0–40 C. Germination was most rapid above 26 C.

At 0–24 C, mycelial growth from plugs of the *M. piriformis* isolates was tough and wiry. Growth was most rapid near 15 C, covering the petri plates (90 mm) in about 2 days. At 0 C, the plates were covered in about 20 days. At each temperature, long gray-white sporangiophores bearing black sporangia were produced. Many microscopic water droplets were produced on the mycelial strands. At 27 C, yeastlike growth developed from the mycelial plugs. When the cultures were transferred to 18 C, seven formed normal filamentous growth in about 4 days. The other eight continued the yeastlike growth habit for 20 days.

Mycelium grew from plugs of the *M. circinelloides* isolates at temperatures of 0–40 C. Growth was most rapid above 26 C and was finer and less tough than that of *M. piriformis* isolates. The colonies appeared yellow-black-brown. Sporangiophores were short and tan-gray and produced tan-gray sporangia. Yeastlike growth often occurred when mycelial plugs were placed at 0 C.

Pathogenicity tests. Both *Mucor* spp. caused decay of stone fruits, apples, pears, oranges, sweet potatoes, tomatoes, carrots, bell peppers, celery, and lettuce at 18 C but not of white potatoes. In addition, *M. piriformis* sometimes caused severe infection of strawberries. On peaches, *M. piriformis* infection differed greatly from *Rhizopus stolonifer* infection. With *M. piriformis*, sporangiophores were very erect, stiff, or wiry even in young infections, whereas the growth of *R. stolonifer* was somewhat prone and cottony (Fig. 3). Both organisms produced black sporangia.

Peach infection. Decay caused by *M. piriformis* isolates developed at most inoculation sites after 4 days on fruit held at 4–24 C (Table 1). After 6 days at 0 C, minute lesions developed at a few inoculation sites. The amount of infection on fruit was greatest at

18 C and slightly less at 13 and 15 C. Initially, lesions were tan and water-soaked, with relatively tough skin. As decay progressed, the flesh became somewhat mushy and watery, extending to the pit, and with pressure the whole fruit collapsed. Gray-white sporangiophores with black sporangia developed within 4 days at temperatures of 13–18 C. A few very short sporangiophores developed in 4 or 6 days in the lesions of fruit held at 24 C and in 10 days in the lesions of fruit held at 0 or 4 C. No sporangiophores appeared from the few lesions that developed at 27 C.

Tomato infection. In ripe tomatoes inoculated with 15 *M. piriformis* isolates, infection was apparent after 1 day at 15 C. The fungus covered most of the fruit, and gray-white sporangiophores with black sporangia were produced within 3 days. At 4 or 27 C, the first symptoms of infection were visible at 3 days (Table 2). Lesions on fruit stored at 4 C were tan and water-soaked. Within 7 days, the fungus had covered a large area of each side of the fruit, and normal fruiting bodies appeared. Decay was complete in about 10 days. Fruit held at 27 C did not decay appreciably. Inoculation sites starting as small tan spots became dried and sunken, with no evidence of aerial mycelial growth or sporangiophores. In fruit held at 0 C, symptoms of infection appeared in about 6 days and decay was usually complete at 15 days. Short sporangiophores with black sporangia were visible.

Infection with the five *M. circinelloides* isolates progressed rapidly at 27 C and was about twice as severe as at 15 C (Table 2). Sporangiophores and sporangia developed at both temperatures within 7 days. In contrast to those of the *M. piriformis* isolates, sporangiophores of the *M. circinelloides* isolates were very short and gray-tan and produced gray-tan sporangia. Fruit were completely decayed within 7 days at both 15 and 27 C, but no infection developed on fruit held at 0 or 4 C for 7 days.

Both groups of isolates caused decay of tomatoes in the four maturity classes within 3 days at 18 C or 5 days at 10 C (Table 3). *M. piriformis* caused considerably more decay than *M. circinelloides*, with less decay in mature-green fruit (22) than in mature fruit. With *M. circinelloides* isolates, mature-green fruit also developed the least decay, and at 18 C the “breakers” decayed less than the pink or red fruit.

TABLE 1. *Mucor piriformis* infection of peaches at various temperatures^a

Holding temperature (C)	Decay after 4 days		Decay after 6 days	
	Infection (%)	Mean diameter (mm)	Infection (%)	Mean diameter (mm)
0	0	0	17	5.1
4	73	5.5	80	11.9
13	87	24.7	100	43.8
15	93	26.5	100	43.9
18	93	35.0	93	50.9
24	80	21.6	87	33.4
27	7	2.1	7	6.3

^aMeans of three tests, five fruit in each test, two inoculation sites each fruit.

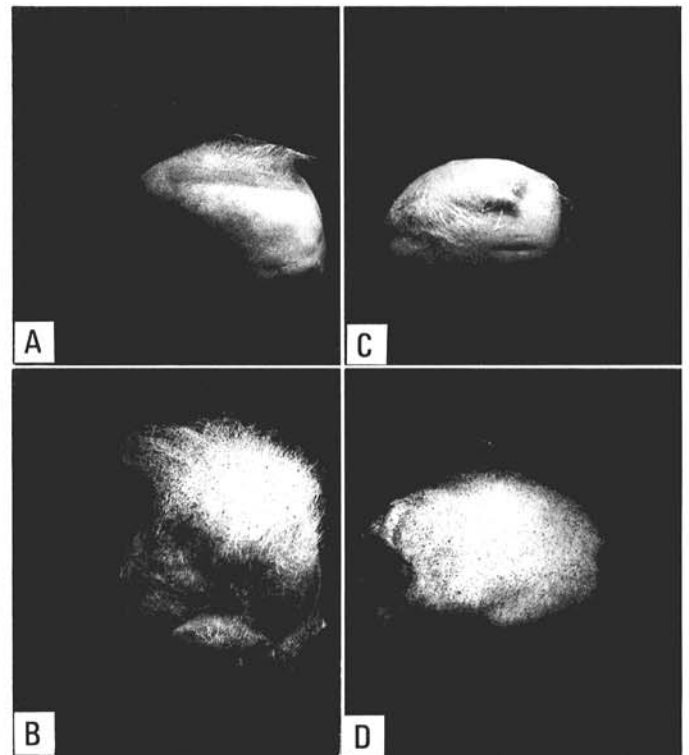


Fig. 3. Comparison of *Mucor* and *Rhizopus* infections of peaches. A, Early stage and B, advanced stage of *Mucor* infection. C, Early stage and D, advanced stage of *Rhizopus* infection.

TABLE 2. Decay of ripe tomatoes by *Mucor* spp. at various temperatures

Holding temperature (C)	Mean diameter (mm) of lesions after 3 days		Mean diameter (mm) of lesions after 7 days	
	<i>M. piriformis</i> ^a	<i>M. circinelloides</i> ^b	<i>M. piriformis</i> ^a	<i>M. circinelloides</i> ^b
0	0	0	6.0	0
4	3.9	0	21.4	0
15	46.7	31.3	80+	80+
27	6.2	65.8	9.4	80+

^aMean of three replicates of five tomatoes inoculated at two sites with 15 isolates.

^bMean of three replicates of five tomatoes inoculated at two sites with five isolates.

TABLE 3. Decay of tomatoes of different maturities by *Mucor* spp.

Maturity	Mean diameter (mm) of lesions at 18 C after 3 days		Mean diameter (mm) of lesions at 10 C after 5 days	
	<i>M. piriformis</i> ^a	<i>M. circinelloides</i> ^b	<i>M. piriformis</i> ^a	<i>M. circinelloides</i> ^b
Mature-green	46.0	4.6	41.5	4.7
Breaker	60.8	12.4	45.3	16.3
Pink	57.5	17.7	46.0	15.6
Red	62.9	22.6	48.3	16.0

^aMean of 15 isolates, three tests, two inoculations each of five fruit.

^bMean of five isolates, two tests, two inoculations each of five fruit.

TABLE 4. Decay of peaches infected with *Mucor* spp. during 6 wk of storage at 5 C

Storage atmosphere	Hand harvested ^a (%)	Commercially harvested ^b (%)
Air	0.0	20.7
Air + IW ^c	15.8	47.7
CA ^d	0.3	32.7
CA + IW	7.4	24.8

^aMean percentage decay of hand-harvested fruit treated with 100 µg/ml of benomyl in water at 46 C for 2.5 min.

^bMean percentage decay of commercially harvested, graded, and hydrocooled fruit.

^cIW = intermittent warming (warmed every 3 wk in air at about 18 C for 2 days, then returned to 5 C).

^dCA = controlled atmosphere (1% O₂ + 5% CO₂).

Pilot storage tests. Peaches that were hand-harvested and dipped in a suspension of 100 µg/ml of benomyl at 46 C developed much less decay than those harvested commercially (Table 4). Earlier studies showed that benomyl-hot water treatment was almost completely ineffective in controlling *Mucor* infection (2). Hand-harvested fruit, however, would be less subject to contamination than commercially harvested fruit. The greater amount of decay in fruit from the commercial packinghouse strongly implies that one cause of losses due to *Mucor* is contamination of the fruit in the packinghouse. Our study also showed that more decay developed in fruit subjected to intermittent warming than in fruit held constantly at 0 C. Controlled-atmosphere storage at 5 C did not reduce decay compared with storage in a normal atmosphere at 5 C.

DISCUSSION

During the marketing of fresh produce, a new pathogen or increased prevalence of an existing pathogen always causes serious problems. This proved to be true of the *Mucor* spp. studied. Although *Mucor* spp. were known to cause decay during marketing of fruits, they have become important pathogens only recently and in certain areas (4-9,13,14,16-21).

Of the two species we studied, *M. piriformis* appears to be more prevalent than *M. circinelloides* and is faster growing and more virulent on the fruits and vegetables we tested. The two species can be easily distinguished, since *M. piriformis* produces tall gray-white sporangiophores with black sporangia and *M. circinelloides*

produces short gray-tan sporangiophores with tan sporangia. The sporangiophores of the latter also are considerably shorter and the sporangiospores are more nearly globose. Although *Rhizopus* spp. could be confused with *Mucor* spp., the *Mucor* spp. used in this study grow at or near 0 C, do not produce rhizoids, and have coarse, wiry vegetative growth.

Many fruits and vegetables should be stored near 0 C to extend their market life. Such a low temperature also inhibits growth of many fungi and bacteria that cause postharvest decay. We found, however, that *M. piriformis* isolates are able to grow and infect produce at 0 C nearly as fast as at 24 C. Since spore germination, vegetative growth, and infection of produce are only slightly delayed by storage at 0 C, this *Mucor* sp. presents a potentially serious postharvest problem. Other workers have also reported *Mucor* spp. that grow and infect produce at 0 C (6,8,16). The *M. piriformis* isolates we studied did not grow normally at 27 C, a temperature at which some types of produce are held for ripening. Isolates of *M. circinelloides*, however, caused decay above 27 C, showing that *Mucor* spp. can infect produce over a wide range of temperatures.

The apparent death of spores and mycelium of the *M. piriformis* isolates in a relatively short period at 27 C is rather surprising, for many fungi grow rapidly at this temperature. To our knowledge, the large increase in spore diameter at the elevated temperatures, the formation of abnormal germ tubes, and the death of sporangiospores have not been reported previously for *Mucor* spp., but abnormal swelling of spores of *Aspergillus* spp. held at elevated temperatures and under CO₂ has been reported (1,23). These workers believe such swelling is caused by a period of active metabolism and growth and is not a passive increase in size due to imbibition of water. We agree with these workers because *M. piriformis* spores, in particular, swelled only when they were on media containing nutrients.

Yeastlike growth of *Mucor* spp. has been reported to be induced by fungicide or nutritional treatments (10,11). We also observed yeastlike growth from most of our isolates, especially when they were placed under temperature stress (27 or 0 C, depending on the isolates). Preliminary tests have shown that yeastlike forms produced at 27 or 0 C are not pathogenic in tomatoes and other fruits (*unpublished*).

We obtained evidence that much of the *Mucor* infection of peaches is due to unsanitary conditions in commercial packing sheds, a condition we suspect prevails throughout the world. Careful handling and sanitation during harvesting, packing, transporting, and marketing peaches, therefore, may be a simple method of controlling this fungus.

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