Mucor Rot—An Emerging Postharvest Disease of Mandarin Fruit Caused by *Mucor piriformis* and other *Mucor* spp. in California

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Abstract

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In recent years, an emerging, undescribed postharvest disease was observed on mandarin fruit after extended storage in California. We collected decayed mandarin fruit from three citrus packinghouses in the Central Valley of California in 2015 and identified this disease as Mucor rot caused by *Mucor* spp. Mucor rot occurred in 11 of the 15 grower lots sampled, and the percentage of Mucor rot in the total decayed fruit varied among affected grower lots, ranging from 3.3 to 93.1% with an average of 49.2%. In total, 197 isolates of *Mucor* spp. were obtained from decayed mandarin fruit and identified based on internal transcribed spacer sequence and morphological characteristics. Of the 197 isolates, 182 (92.4%) were identified as *Mucor piriformis*, 7 (3.6%) were *M. circinelloides* (6 *M. circinelloides* f. *lusitanicus* and 1 *M. circinelloides* f. *circinelloides*), 4 (2%) were *M. racemosus* f. *racemosus*, 3 (1.5%) were *M. hiemalis*, and 1 (0.5%) was *M. mucedo*. All species grew at 0 and 5°C, except *M. circinelloides*, which did not grow at 0°C. Mycelial growth was arrested at 27°C for *M. piriformis*; 35°C for *M. racemosus* f. *racemosus*, *M. circinelloides* f. *lusitanicus*, *M. hiemalis* and *M. mucedo*; and 37°C for *M. circinelloides*

f. *circinelloides*. Optimal mycelial growth occurred at 20°C for *M. piriformis* and *M. mucedo*, 25°C for *M. racemosus* f. *racemosus* and *M. hiemalis*, 27°C for *M. circinelloides* f. *lusitanicus*, and 30°C for *M. circinelloides* f. *circinelloides*. *M. piriformis* grew significantly faster than the other four species at 5 and 20°C, and *M. mucedo* was the slowest in growth among the five species. Sporangiospores of *M. piriformis*, *M. racemosus* f. *racemosus*, and *M. hiemalis* germinated at both 5 and 20°C. *M. circinelloides* germinated at 20°C but did not germinate at 5°C after incubation for 48 h. All five *Mucor* spp. caused decay on mandarin fruit inoculated with the fungi, and the lesion size caused by *M. piriformis* was significantly larger than that caused by other species at both 5 and 20°C. Our results indicated that Mucor rot in mandarin fruit in California is caused by *Mucor* spp. consisting of *M. piriformis*, *M. circinelloides*, *M. racemosus* f. *racemosus*, *M. hiemalis*, and *M. mucedo*, with *M. piriformis* being the dominant and most virulent species. Previously, *M. racemosus* was reported on citrus. This is the first report of Mucor rot in citrus caused by *M. piriformis*, *M. circinelloides*, *M. hiemalis*, and *M. mucedo*.

Citrus is one of the most important fruit crops in the world and has grown commercially in more than 140 countries located in tropical and subtropical areas (Terol et al. 2007). In the United States, California is the leading producer of fresh-market citrus, accounting for about 76% of the production in 2013 (CDFA-CASS 2014). Among various types of citrus produced in the region, California leads in navel orange production and also produces significant percentages of the nation's Valencia orange, lemon, and grapefruit. However, during the last decade, there has been a constant decrease in consumption of "difficult-to-peel" citrus fruit such as orange and grapefruit, accompanied by increasing consumption and demand for "easy-to-peel" mandarin fruit (Ladaniya 2008; USDA-FAS 2016). The biggest change in California's citrus industry in the past decade has been the increasing production of mandarin (Webber et al. 2014). The total acreage of orange orchards decreased from 75,700 to 68,400 ha from 2004 to 2014, while that of mandarin has steadily increased from

7,200 to 18,600 ha in this period (CDFA-CASS 2014), with a production value in 2014 exceeding \$500 million (USDA-NASS 2015). Most mandarin fruit are marketed fresh and fill the harvest niche between Valencia orange and other summer fruit.

In California, at harvest, mandarin fruit are hand-picked and placed into plastic bins, which are then transported to packinghouses. Traditionally, mandarin fruit are presorted and packed into cartons or bags for marketing shortly after harvest. As the acreage of mandarin continues to expand, the industry has been exploring ways to expand the current marketing window. One such approach is to store mandarin fruit in a cold facility after harvest for a certain period of time and pack them at a later time. However, extended storage of fruit after presorting may predispose the fruit to the development of fruit rots because all types of citrus fruit are susceptible to postharvest fruit rots. Postharvest fruit rots caused by various fungal pathogens contribute to the most significant losses to the citrus industry during degreening, storage, transportation, and marketing (Akhtar et al. 2013; Smilanick et al. 2006).

Several postharvest fruit rots commonly affect citrus (Snowdon 1990). Postharvest diseases of citrus fruit can result from three types of infections: infections that develop during the growing season and continue to cause postharvest problems, infections that initiate in the field but remain latent before harvest and become active to develop decay symptoms only after harvest, and infections that initiate at wounds during harvest and postharvest handling (Arpaia et al. 2014). Green mold caused by *Penicillium digitatum* (Pers.) Sacc., blue mold caused by *P. italicum* Wehmer, and sour rot caused by *Geotrichum citri-aurantii* (Ferraris) E. E. Butler are generally considered the most common postharvest diseases in citrus worldwide (Akhtar et al. 2013; Bhattarai et al. 2013; McKay et al. 2012; Smilanick and Mansour 2007; Smilanick et al. 2006). However, the occurrence of postharvest fruit rots of citrus can be affected by both fruit growing conditions in the field and postharvest storage and handling practices (Arpaia et al. 2014; Smilanick et al. 2008). Citrus types and cultivars

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may exhibit varying susceptibility to different postharvest diseases. Although numerous studies on postharvest diseases of citrus have been documented, most of the research was conducted on orange and lemon and little research has been conducted regarding the postharvest fungal diseases of mandarin fruit in California.

In 2013, some citrus packinghouses in the Central Valley of California, which is a major region for citrus production, indicated that, in recent years, they had experienced an unknown postharvest disease on mandarin fruit, particularly on the fruit that had been stored for an extended period of time after harvest at low temperatures. During the 2014–15 storage season, we collected decayed mandarin fruit from three commercial citrus packinghouses in the region and confirmed that the unknown disease was caused by *Mucor* spp. Thus, the objectives of this study were to (i) describe *Mucor* rot on mandarin fruit caused by *Mucor* spp., (ii) identify *Mucor* spp. associated with fruit rot of mandarin in California, (iii) conduct comparative studies on the biology of different *Mucor* spp. associated with mandarin, and (iv) test pathogenicity of *Mucor* spp. on mandarin fruit.

Materials and Methods

Symptoms and occurrence of *Mucor* rot. Surveys for *Mucor* rot in stored mandarin fruit were conducted in the Central Valley of California during mid-January to the end of April in 2015. Decayed mandarin fruit were sampled from three packinghouses. At each sampling time, approximately 30 to 60 decayed fruit from one grower lot (one grower lot represents the fruit from the same orchard block) were arbitrarily sampled from storage bins in the packinghouses, and two to three grower lots were sampled each week. In total, 553 decayed fruit from 15 grower lots were sampled. Decayed fruit were placed individually on citrus fruit-packing trays and transported to the laboratory on the same day they were sampled. Samples were sorted by symptoms and the presence of wounds. Color and texture (firm, soft, or watery) of the decayed area were also recorded. All decayed fruit were photographed before pathogen isolation in order to correlate causal agents with the disease symptoms they incited.

Isolation and identification of causal agents. Identification of fungal pathogens was initially made based on disease symptoms exhibited on the rind of decayed fruit, and morphology of fungi under a microscope if sporulation or fruiting bodies of the pathogens were present on the fruit. All decayed fruit with sporulation similar to *Mucor* spp. were subjected to isolation of causal agents. To isolate causal agents from decayed fruit, decayed fruit were surface sterilized by spraying them with 70% ethanol and allowed to air dry in a fume hood. Small fragments of the rind were removed using a sterile scalpel from the margin of decayed and healthy tissue and placed onto petri dishes containing water agar. The plates were incubated at 20°C for 5 to 7 days, allowing fungal pathogens to grow. Mycelia actively growing on the edge of the colonies on the water agar were transferred onto potato dextrose agar (PDA; Difco Laboratories, Detroit). The PDA plates were then incubated at 20°C for another 4 to 5 days. Initial identification of putative *Mucor* isolates to the genus level was based on the descriptions of Schipper (1973, 1975, 1976, 1978). In total, 197 putative *Mucor* isolates were obtained from decayed mandarin fruit.

After the preliminary morphological analysis, single-sporangiospore isolation was conducted for all *Mucor* isolates. Sporangiospores from 1-week-old PDA cultures were scraped off using a sterile scalpel and suspended in 1 ml of sterile distilled water with 0.1% of Tween 20. An aliquot of 50 μ l of sporangiospore suspension was spread on water agar in a petri dish. After incubation at 20°C for 1 to 2 days, single germinated sporangiospores were transferred onto new PDA plates and incubated at 20°C for another 4 to 5 days for mycelial development. Mycelial plugs excised using a sterile scalpel were transferred into a 2-ml vial filled with 15% glycerol and stored at –80°C.

DNA extraction, polymerase chain reaction amplification, and sequencing of *Mucor* spp. All *Mucor* isolates were subjected to DNA sequencing for identification to the species level (Walther et al. 2013). Genomic DNA was extracted from mycelium of *Mucor* isolates grown on PDA using DNeasy Mini Spin Columns (Qiagen, Valencia, CA) according to the manufacturer's protocol. Resulting DNA solutions were used for sequencing as described below.

The internal transcribed spacer (ITS) region was amplified for all *Mucor* isolates using the primer pair ITS1/ITS4 (White et al. 1990). Polymerase chain reaction (PCR) was performed as described previously, with modifications (White et al. 1990). Briefly, PCR amplifications were performed in a 10- μ l volume containing 10 ng of fungal genomic DNA, 0.2 μ M each primer, and 5.0 μ l of GoTaq PCR mixture (Promega Corp., Madison, WI). PCR was carried out in a S1000 thermal cycler (Bio-Lad Life Science Research Group, Hercules, CA) with the following conditions: an initial preheating for 2 min at 95°C; followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min; and a final extension at 72°C for 5 min.

PCR products were purified using a GeneJet purification kit (Thermo Fisher Scientific, Pittsburgh) according to manufacturer's protocols. Purified PCR products were then directly sequenced in both directions using BigDye Terminator v3.1 Cycle Sequencing Kit in a 3130XL Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA). Sequences were analyzed using BioEdit Sequence Alignment Editor, v7.1.11 (Hall 1999). The ITS sequences of the 197 *Mucor* isolates from mandarin fruit were subjected to a Mega BLAST search on the National Center for Biotechnology (NCBI) website (<http://www.ncbi.nlm.nih.gov/>) to find highly similar sequence data, and then were compared with the sequences of ex-type strains of the known *Mucor* spp. reported previously (Walther et al. 2013).

Morphological characteristics. Two isolates each of *Mucor piriformis* (X1816 and X1944), *M. racemosus* f. *racemosus* (X1650 and X2081), and *M. hiemalis* (X1946 and X2121); three isolates of *M. circinelloides* (X1785, X1899, and X1935); and one isolate of *M. mucedo* (X2142) that were identified by ITS sequencing were selected for morphological characterization. To compare morphological characteristics of *Mucor* spp. from mandarin fruit with the known taxa of *Mucor* spp., all isolates were cultured on PDA and beer wort agar (BWA) following the procedure of Schipper (1973, 1975, 1976, 1978). For all isolates, 1-week-old cultures incubated at 20°C in darkness were used for measuring the dimensions of sporangiospores, sporangia, and columellae. Averages or size ranges of the measurements for each organ were calculated from at least 50 measurements for each isolate. Experiments were performed twice. Morphological characteristics on BWA were observed daily, recorded, and compared with the morphological descriptions of the known *Mucor* spp. (Schipper 1973, 1975, 1976, 1978).

In addition, to compare potential differences in culture morphology, one representative isolate each of four *Mucor* spp.—X1816 (*M. piriformis*), X1650 (*M. racemosus* f. *racemosus*), X2121 (*M. hiemalis*), and X2142 (*M. mucedo*)—and two isolates of *M. circinelloides*—X1785 (*M. circinelloides* f. *lusitanicus*) and X1899 (*M. circinelloides* f. *circinelloides*)—were cultured on four different media: (i) PDA, (ii) BWA (Schipper 1973, 1975, 1976, 1978), (iii) synthetic *Mucor* agar (SMA) (Michailides 1991), and (iv) orange juice agar (OJA). OJA was made based on apple juice agar (Xiao and Sittou 2004) by substituting apple juice with orange juice. The ingredients of OJA were as follows: 200 ml of orange juice (Sunnyside Farms, Turlock, CA), 3.0 g of CaCO₃, 15 g of agar, and 800 ml of deionized water.

Agar plugs (5 mm) containing actively growing mycelium were excised with a sterile cork borer from the edge of 2- to 3-day-old SMA cultures at 20°C, and one such mycelial plug was placed upside-down in the center of each plate. Three replicate plates of each medium were used for each isolate. Colony diameter was measured in two perpendicular directions after 48 h of incubation at 20°C in the dark and means were calculated after subtracting the diameter of the original plug (5 mm). Each plate was photographed at the end of the experiments. Experiments were performed twice.

Mycelial growth in response to temperature. Radial mycelial growth of *Mucor* spp. at different temperatures was determined on SMA in 90-mm petri dishes (Michailides 1991), with slight modifications. Briefly, an agar plug (5 mm in diameter) containing actively growing mycelium prepared as described above was placed upside-down in the center of a new SMA plate. Two isolates each of

M. piriformis, *M. racemosus* f. *racemosus*, and *M. hiemalis*; seven isolates of *M. circinelloides* (six *M. circinelloides* f. *lusitanicus* and one *M. circinelloides* f. *circinelloides*); and one isolate of *M. mucedo* were used for this test. Three replicate plates were used for each isolate. The plates were incubated in the dark at 0, 5, 10, 15, 20, 25, 27, 30, 35, 37, and 40°C. The colony diameter in each plate was measured as described above after 24 and 48 h of incubation for all temperatures except 0°C. Because all *Mucor* isolates grew slowly at 0°C, the colony diameter was measured after 3 and 6 days for isolates X1816 and X1944 (*M. piriformis*) and 1 and 2 weeks for the isolates of other species. Radial mycelial growth rate of each isolate was then calculated and expressed as millimeters per day. Experiments were performed twice.

Germination of sporangiospores. To determine germination of sporangiospores, a 50- μ l aliquot of the sporangiospore suspension of each isolate, prepared as described above, was pipetted onto a 90-mm petri dish containing SMA and spread evenly on the surface. Three replicate plates for each isolate were incubated in the dark at 5°C for 48 h or at 20°C for 14 h. Because isolate X2142 (*M. mucedo*) did not germinate on SMA after respective incubation conditions, germination was then tested on OJA following the same procedure. At the end of the incubation period, at least 100 sporangiospores in each plate were examined under a microscope ($\times 10$) and the average percentage of germination of sporangiospores was calculated for each isolate at each temperature. Experiments were performed twice.

Pathogenicity tests. Two isolates each of *M. piriformis* (X1816 and X1944) and *M. circinelloides* (X1785 [*M. circinelloides* f. *lusitanicus*] and X1899 [*M. circinelloides* f. *circinelloides*]) and one isolate each of *M. racemosus* f. *racemosus* (X1650), *M. hiemalis* (X2121), and *M. mucedo* (X2192) were used in this study.

To make the sporangiospore suspension, 1- to 2-week-old PDA cultures of the isolates that were incubated at 20°C in the dark were flooded with sterile distilled water and scraped with a sterile rod, and the resultant sporangiospore suspension was filtered through autoclaved gauze. Number of sporangiospores in the suspension was estimated under a microscope with the aid of a hemocytometer and diluted to a concentration of 1.0×10^5 sporangiospores/ml.

Mandarin fruit ('W. Murcott') that were not treated with any post-harvest treatments and provided by a local packinghouse were used for pathogenicity tests. Prior to the experiments, fruit were surface sterilized by dipping them into 0.5% sodium hypochlorite for 2 min, rinsed with sterile water three times, and allowed to air dry in a fume hood. The fruit were then placed individually on the base of 35-mm petri dishes so that each fruit was held steady for inoculation. Two different inoculation methods were used in this study.

Wound inoculation. A wound (3 mm in diameter and 7 mm in depth) was made approximately 1.5 cm away from the stem end of the fruit using a sterile needle inserted into the rind. An aliquot of 10 μ l of the sporangiospore suspension of the isolate was pipetted into each wound. Ten fruit were used for each isolate. The fruit treated with sterile water were used as noninoculated controls. After inoculation, the inoculated fruit held on the base of petri dishes were then placed into clear plastic containers, which were then covered. Paper towels saturated with sterile distilled water were placed on the bottom of the containers to establish high relative humidity in the containers, and the containers were incubated in the dark at 5°C for 3 weeks or at 20°C for 11 days. After incubation, disease symptoms on the fruit were recorded. All fruit were then cut vertically in half, passing through the wound, using a sterile cutter, and the disease symptoms inside of the fruit were also recorded. At the end of the experiment, three decayed fruit inoculated with each isolate were arbitrarily chosen in attempts to reisolate the pathogen. Single-sporangiospore isolation was conducted for each recovered fungus, followed by morphological identification and DNA sequencing for speciation as described above, to fulfill Koch's postulates. Experiments were performed twice.

Cut-fruit inoculation. Preliminary experiments indicated that decay lesions developed on wound-inoculated fruit were often irregular on the rind of the fruit, making lesion size measurement to quantify the differences in virulence among *Mucor* spp. difficult. Thus, we

used the cut-fruit assay as described below to quantify the potential differences in virulence among the isolates of *Mucor* spp. tested in this study.

Approximately one-third of each fruit was sliced off vertically in the direction from the stem end to the stylar end of the fruit using a sterile cutter. Each cut fruit was placed with the cut-side up on the base of a 35-mm petri dish in order to hold the fruit steady. An aliquot of 5 μ l of the sporangiospore suspension of an isolate was placed at the center of the cut surface of the cut fruit using a micropipette. Ten replicate fruit were used for each isolate. The fruit treated with sterile water were used as noninoculated controls. After inoculation, the fruit were transferred into plastic containers with the same conditions as described above. The containers were incubated in the dark at 5°C for 9 days and at 20°C for 2 days. The fruit inoculated with isolate X1899 (*M. circinelloides* f. *circinelloides*) showed no or very small decay lesions 9 days after inoculation at 5°C; therefore, an additional 10-day incubation time was added for these fruit and, thus, the result of isolate X1899 was excluded from the statistical analysis described below. On the cut fruit, *Mucor* rot developed circular lesions. At the end of incubation, the presence of *Mucor* rot symptoms on each fruit was recorded, and lesion size on the cut surface was measured for each fruit. Experiments were performed twice.

Statistical analysis. All statistical analyses were conducted using SAS (version 9.4; SAS Institute, Inc., Cary, NC). The percentage data from the germination tests were arcsine-square root transformed prior to analysis. Analysis of variance using PROC GLM in SAS was performed to determine if data across the runs of each experiment could be pooled. The test indicated that there were no interactions between run and variables ($P > 0.05$), and thus all data were pooled and further analyzed using PROC GLM, and mean separations were made by Tukey's honestly significant difference multirange tests.

Results

Symptoms, prevalence, and incidence of the disease. On decayed fruit collected from packinghouses, *Mucor* rot appeared to develop from infections through puncture wounds or cracks on the rind of the fruit (Fig. 1). In the early stage of symptom development, lesions were quasicircular or irregular, light to dark brown, and soft and watery, and sporangiophores of *Mucor* spp. protruded from the wounds or cracks (Fig. 1). At the advanced stage of disease development, infected fruit generally were completely covered with masses of white or white to gray shiny, erect sporangiophores, on the top of which black or pale brown sporangia were evident, and decayed tissues were very watery (Fig. 1). *Mucor* rot symptoms were also observed on decayed fruit intermixed with sporulation of other decay pathogens such as *Penicillium* or *Geotrichum* spp.

Mucor rot was observed in fruit sampled from all three packinghouses. *Mucor* rot occurred in 11 of the 15 grower lots sampled, and the percentage of *Mucor* rot in the total decayed fruit varied greatly among affected grower lots, ranging from 3.3 to 93.1%, with an average of 49.2% (Table 1). On average, *Mucor* rot accounted for 1.7, 52.0, and 54.6% of the decayed fruit for the samples collected from packinghouses I, II, and III, respectively (Table 1).

***Mucor* spp. identified by ITS sequencing.** Based on the ITS sequences of the 197 *Mucor* isolates, the Mega BLAST search on NCBI (<http://www.ncbi.nlm.nih.gov/>) showed that 182 (92.4%), 4 (2%), 3 (1.5%), and 1 isolate (0.5%) had ITS sequences with high similarities to those of ex-type strains of *M. piriformis* (accession number JN206028; >97%), *M. racemosus* f. *racemosus* (accession number JN205898; >99%), *M. hiemalis* (accession number JN206129; >99%), and *M. mucedo* (accession number JN206085; >96%), respectively; thus, they were identified as *M. piriformis*, *M. racemosus* f. *racemosus*, *M. hiemalis*, and *M. mucedo*, respectively. Six isolates (X1785, X1935, X1945, X1949, X2158, and X2159) had ITS sequences with 97% similarity to that of *M. circinelloides* f. *lusitanicus* (accession number JN205980), and one isolate (X1899) had 98% similarity in ITS sequence to that of *M. circinelloides* f. *circinelloides* (accession number JN205942). GenBank accession numbers for sequences of representative isolates of the five species of *Mucor* from mandarin fruit are listed in Supplementary Table S1.

Morphological characteristics. Schipper (1973, 1975, 1976, 1978) described morphological characteristics of *Mucor* spp. based on the cultures on BWA incubated at 20°C in the dark; therefore, in the present study, all observations of morphological characteristics of the five *Mucor* spp. identified by ITS sequencing were made under the same conditions (Table 2; Fig. 2).

Isolates X1816 and X1944, identified as *M. piriformis* based on ITS sequences, had a colony up to 45 mm in height, white, and aromatic; sporangiophores bore droplets at the basal part, unbranched at first, then branched with short branches; sporangia were blackish and variable in size, ranging from 78 to 249 by 81 to 279 µm; columellae variable in shape and size, the large columellae obovoid or pyriform, the smaller columellae cylindrical-ellipsoidal or pyriform, ranging from 45 to 186 by 75 to 207 µm; sporangiospores ellipsoidal or subglobose to globose, 4.7 to 10.9 by 3.0 to 9.5 µm. These morphological characteristics were in agreement with the descriptions of *M. piriformis* (Schipper 1975).

Isolates X1650 and X2081, identified as *M. racemosus* f. *racemosus* based on ITS sequences, had a colony up to 20 mm in height, white to grayish; sporangiophores branched in a mixed sympodial and monopodial fashion, sporangia pale brown to pale gray, variable in size, ranging from 25 to 90 by 25 to 89 µm; columellae variable in shape and size, obovoid, ellipsoidal, cylindrical-ellipsoidal to subglobose or pyriform, usually with truncate base, ranging from 14 to 55 by 14 to 43 µm; sporangiospores ellipsoidal to subglobose, 4.3 to 10.1 by 3.5 to 7.9 µm, clamydospores numerous in sporangiospores and even in a few columellae. These morphological characteristics were in agreement with the descriptions of *M. racemosus* f. *racemosus* (Schipper 1976).

Isolates X1785 and X1935, identified as *M. circinelloides* f. *lusitanicus* based on ITS sequences, had a colony up to 4 mm in height, white to pale gray; sporangiophores repeatedly branched sympodially, infrequently circinate; sporangia faintly yellowish, later brownish, 26 to 88 by 29 to 82 µm; columellae globose to subglobose, 15 to 50 by 15 to 42 µm; sporangiospores variable in size and shape, ellipsoidal, infrequently large and bizarre, 4.3 to 8.6 by 2.7 to 6.4 µm. These morphological characteristics were in agreement with the descriptions of *M. circinelloides* f. *lusitanicus* (Schipper 1976).

Isolate X1899, identified as *M. circinelloides* f. *circinelloides* based on ITS sequence, had a colony up to 5 mm in height, white to grayish; sporangiophores branched sympodially with long and short branches, the latter circinate; sporangia pale brown to pale gray, ranging from 31 to 87 by 30 to 87 µm; columellae obovoid to ellipsoidal in the larger sporangia, globose in the smaller sporangia, 17 to 47 by 17 to 48 µm; sporangiospores ellipsoidal to subglobose, 4.2 to 10.4 by 2.7 to 5.6 µm, clamydospores few in number. These morphological characteristics were in agreement with the descriptions of *M. circinelloides* f. *circinelloides* (Schipper 1976).

Isolates X1946 and X2121, identified as *M. hiemalis* based on ITS sequences, had a colony up to 15 mm in height, buff yellow; sporangiophores branched sympodially; sporangia yellowish to pale brown, 44 to 79 by 43 to 94 µm; columellae subglobose to ellipsoidal with truncate base, 22 to 50 by 22 to 45 µm; sporangiospores subglobose to ellipsoidal, sometimes flattened at one side, containing scattered granules, 4.7 to 9.4 by 2.5 to 6.6 µm. These morphological characteristics were in agreement with the descriptions of *M. hiemalis* (Schipper 1973).

Isolate X2142, identified as *M. mucedo* based on ITS sequence, had a colony up to 20 mm in height, white grayish; sporangiophores usually unbranched; sporangia whitish to yellowish at first, light brownish to gray at maturity, 51 to 153 by 55 to 138 µm; columellae obovoid to ellipsoidal, 48 to 136 by 33 to 85 µm; sporangiospores broadly ellipsoidal, frequently containing “cloudy” granules, 9.0 to 16.0 by 5.2 to 10.2 µm. These morphological characteristics were in agreement with the descriptions of *M. mucedo* (Schipper 1975).

Mycelial growth on different media. The five *Mucor* spp. were able to grow on the four media at 20°C with varying mycelial growth rates (Table 3; Fig. 3). Media significantly affected mycelial growth of *Mucor* spp. Regardless of media, *M. piriformis* (X1816) grew significantly faster than the other four species, and *M. circinelloides* f. *lusitanicus* (X1785) grew faster than *M. racemosus* f. *racemosus*, *M. hiemalis*, *M. mucedo*, and *M. circinelloides* f. *circinelloides* (X1899) (Table 3). SMA was the best for mycelial growth of *M. piriformis* and *M. circinelloides* f. *circinelloides* (X1899), while OJA was the best for mycelial growth of *M. racemosus* f. *racemosus*, *M. hiemalis*, and *M. circinelloides* f. *lusitanicus* (X1785). BWA

Fig. 1. Symptoms of *Mucor* rot caused by *Mucor piriformis* on mandarin fruit collected from citrus packinghouses.

Table 1. Percentages of *Mucor* rot in decayed mandarin fruit sampled from 15 grower lots in three packing houses in 2015

Fruit and rot	Date, packinghouse (I to III), grower lots (A to O)														
	17 March		24 March			27 March			8 April		14 April		21 April		
	I		II			III			III		II		I		
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Number of fruit	60	30	59	30	30	30	45	30	30	29	60	30	30	30	30
Mucor rot (%)	5.0	0	78.0	50.0	63.3	0	26.7	73.3	80.0	93.1	38.3	30.0	0	0	3.3

was the best for mycelial growth of *M. mucedo* (Table 3). In general, for all isolates tested, mycelial growth on PDA was relatively poor while that on OJA was relatively good (Table 3; Fig. 3). Colony color of isolates X1650 (*M. racemosus* f. *racemosus*), X1899 (*M. circinelloides* f. *circinelloides*), and X2142 (*M. mucedo*) was media dependent (Fig. 3). For isolate X1650 (*M. racemosus* f. *racemosus*), the colony appeared slightly brown on PDA, white on SMA, and white-olive green on BWA (Fig. 3). For isolate X1899 (*M. circinelloides* f. *circinelloides*), the colony appeared dense on PDA and light gray in the center on BWA (Fig. 3). For isolate X2142 (*M. mucedo*), the colony appeared light brown on PDA and light gray in the center on BWA (Fig. 3).

Mycelial growth at different temperatures. Radial mycelial growth rates in response to different temperatures varied among the

five *Mucor* spp. (Fig. 4). Except for the seven isolates of *M. circinelloides*, all isolates grew at 0°C (Fig. 4). Except for the two isolates of *M. piriformis*, all isolates grew at 30°C. Isolate X1899 (*M. circinelloides* f. *circinelloides*) was the only one that was able to grow at 35°C (Fig. 4). Optimal temperature for mycelial growth was 20°C for *M. piriformis* and *M. mucedo*, 25°C for *M. racemosus* f. *racemosus* and *M. hiemalis*, 27°C for the six isolates of *M. circinelloides* f. *lusitanicus*, and 30°C for isolate X1899 (*M. circinelloides* f. *circinelloides*) (Fig. 4). At 27°C, the two isolates of *M. piriformis* grew in a yeast fashion but they stopped growing on the third day of experiment, and they did not resume mycelial growth when incubated at 20°C after removal from 27°C. The six isolates of *M. circinelloides* f. *lusitanicus* showed a similar growth curve at each temperature; thus, only the result of isolate X1785 was shown in Figure 4. *M. racemosus* f. *racemosus*, *M. hiemalis*, *M. mucedo*, and

Table 2. Dimension of sporangiospores, sporangia, and columellae of the five *Mucor* spp. on potato dextrose agar (PDA) and beerwort agar (BWA) incubated at 20°C in the dark for 1 week^y

Species, isolate ^z	Sporangiospores				Sporangia		Columellae	
	PDA		BWA		PDA	BWA	PDA	BWA
	Avg (μm)	Range (μm)	Avg (μm)	Range (μm)	Range (μm)	Range (μm)	Range (μm)	Range (μm)
<i>Mucor piriformis</i>								
X1816	8.1 by 5.5	5.0–12.5 by 3.2–11.4	8.1 by 5.9	4.9–10.9 by 3.6–8.0	73–241 by 80–279	78–276 by 93–274	59–203 by 55–170	75–207 by 47–186
X1944	8.8 by 6.2	5.4–12.9 by 3.0–12.1	7.3 by 5.3	4.7–10.7 by 3.0–9.5	80–249 by 81–237	113–279 by 110–288	57–207 by 45–163	79–203 by 45–181
<i>M. racemosus</i>								
X1650	7.6 by 5.8	5.0–9.9 by 3.9–8.1	6.8 by 5.6	4.3–8.4 by 3.5–7.1	44–83 by 38–82	25–80 by 25–72	23–53 by 14–38	14–43 by 14–30
X2081	7.1 by 5.7	4.9–9.4 by 3.8–7.4	7.1 by 6.0	4.9–10.1 by 3.8–7.9	39–79 by 39–78	31–90 by 32–89	22–52 by 18–38	21–55 by 18–43
<i>M. circinelloides</i>								
X1785	6.6 by 4.6	4.0–11.1 by 2.9–9.0	6.2 by 4.1	4.3–8.4 by 2.7–6.4	33–87 by 32–79	26–88 by 29–82	13–52 by 12–43	15–50 by 15–39
X1899	6.0 by 4.5	3.4–11.4 by 3.4–8.8	5.8 by 4.0	4.2–10.4 by 2.7–5.6	39–94 by 41–92	31–87 by 30–87	21–44 by 20–43	17–47 by 17–48
X1935	6.8 by 4.5	4.8–9.7 by 3.0–7.2	6.1 by 3.8	4.3–8.6 by 3.0–5.9	32–75 by 30–76	35–76 by 34–80	21–51 by 18–48	21–48 by 18–42
<i>M. hiemalis</i>								
X1946	7.2 by 5.1	5.0–9.8 by 3.2–7.5	6.8 by 4.3	4.7–9.4 by 2.5–6.6	44–79 by 47–85	45–90 by 48–90	28–44 by 26–39	22–50 by 23–45
X2121	6.9 by 4.8	4.6–9.3 by 3.2–7.4	6.5 by 3.9	4.9–8.5 by 3.0–5.3	49–75 by 49–79	37–94 by 43–94	27–47 by 27–41	22–43 by 22–40
<i>M. mucedo</i>								
X2142	14.5 by 7.9	6.7–18.3 by 6.0–10.3	12.6 by 7.5	9.0–16.0 by 5.2–10.2	65–170 by 75–177	51–153 by 55–138	65–175 by 42–121	48–136 by 33–85

^y Avg = average and Range = size range.

^z X1785 and X1935, *M. circinelloides* f. *lusitanicus*; X1899, *M. circinelloides* f. *circinelloides*.

Fig. 2. Photomicrographs of representative sporangiospores, sporangia, and columellae of five *Mucor* spp. Microscopy was performed on cultures grown on beerwort agar. The height of each micrograph is scaled to 50 μm for sporangiospores and 150 μm for sporangia and columellae. X1650, *Mucor racemosus* f. *racemosus*; X1785, *M. circinelloides* f. *lusitanicus*; X1899, *M. circinelloides* f. *circinelloides*.

the six isolates of *M. circinelloides* f. *lusitanicus* did not grow at 35°C (Fig. 4) but they resumed mycelial growth when incubated at 20°C after removal from 35°C. Isolate X1899 (*M. circinelloides* f. *circinelloides*) did not grow at 40°C but resumed mycelial growth when incubated at 20°C.

Radial mycelial growth rates at two temperatures were compared among the five *Mucor* spp. isolates (Table 4). These two temperatures (5 and 20°C) were chosen because they represent the temperatures used for storage of mandarin fruit in commercial packinghouses and that of retail stores, respectively. At these two temperatures, *M. piriformis* grew significantly faster than the other species, and *M. mucedo* was the most slow-growing one among the five species (Table 4).

Germination rate. Germination of sporangiospores was examined on SMA at two temperatures (5 and 20°C). Sporangiospores of *M. piriformis*, *M. racemosus* f. *racemosus*, and *M. hiemalis*

germinated at both 5 and 20°C (Table 5). *M. circinelloides* germinated at 20°C but did not germinate at 5°C after incubation for 48 h (Table 5). When the incubation was extended to 96 h at 5°C, germination rate was 4.9, 1.6, and 5.5% for isolates X1785 (*M. circinelloides* f. *lusitanicus*), X1899 (*M. circinelloides* f. *circinelloides*), and X1935 (*M. circinelloides* f. *lusitanicus*), respectively. *M. mucedo* (X2142) did not germinate on SMA at either 5 or 20°C, and no germination was observed even after 72 and 24 h at 5 and 20°C, respectively; however, the isolate germinated on OJA at both temperatures (Table 5). At 20°C, germination rates of the two isolates of *M. racemosus* f. *racemosus* were significantly higher than those of the others, while germination rates of the two isolates of *M. piriformis* were significantly higher than those of the others at 5°C (Table 5).

Pathogenicity. On the wound-inoculated mandarin fruit, all five *Mucor* spp. were able to cause decay (Fig. 5), and all inoculated fruit (100%) developed Mucor rot symptoms at both 5 and 20°C,

Table 3. Mycelial growth of the five *Mucor* spp. on four different media at 20°C for 48 h in the dark

Media ^z	Colony diameter (mm) ^x					
	<i>Mucor piriformis</i>	<i>M. racemosus</i>	<i>M. circinelloides</i> ^y		<i>M. hiemalis</i>	<i>M. mucedo</i>
	X1816	X1650	X1785	X1899	X2121	X2142
PDA	65.4 A c	33.3 E d	42.2 B c	36.3 D c	38.1 C d	37.7 C c
SMA	78.5 A a	41.1 E c	50.2 B b	47.4 C a	42.2 D c	33.6 F d
BWA	71.2 A b	45.5 CD b	49.6 B b	34.8 E d	45.1 D b	46.2 C a
OJA	66.4 A c	52.1 C a	56.1 B a	43.2 D b	51.9 C a	42.8 D b

^x Values followed by the same uppercase letter within the same row or the same lowercase letter within the same column are not significantly different ($P = 0.05$) according to Tukey's honestly significant difference tests.

^y X1785, *M. circinelloides* f. *lusitanicus* and X1899, *M. circinelloides* f. *circinelloides*.

^z PDA = potato dextrose agar, SMA = synthetic Mucor agar, BWA = beer wort agar, and OJA = orange juice agar.

Fig. 3. Colony morphology of the five *Mucor* spp. on four different media incubated in the dark at 20°C for 48 h. PDA = potato dextrose agar, SMA = synthetic Mucor agar, BWA = beer wort agar, and OJA = orange juice agar. X1650, *Mucor racemosus* f. *racemosus*; X1785, *M. circinelloides* f. *lusitanicus*; X1899, *M. circinelloides* f. *circinelloides*.

regardless of the species. No decay symptoms were observed on non-inoculated control fruit.

The following descriptions of the disease symptoms were made on mandarin fruit that were wounded, inoculated with *Mucor* spp., and incubated at 5°C.

Symptoms on the fruit inoculated with *M. piriformis* started to appear about 1 week after inoculation. Infected tissues of the rind became soft and light to dark brown. As infections advanced, the lesions around the wound formed a quasicircular or irregular shape, slightly shrunk inwardly, forming wrinkles on the surface, and sporangiophores protruded through the wound. When the decayed fruit was cut, the fruit flesh appeared to be completely rotten, light to dark brown, very soft or watery, and separated easily from the healthy tissue, and sporangiophores often were present in the empty space inside the fruit (Fig. 5A and G).

Symptoms on the fruit inoculated with *M. racemosus* f. *racemosus* appeared 2 weeks after inoculation. Only a small lesion (less than 10 mm in diameter) developed around the wound and became soft and dark brown. White sporangiophores protruded through the

wound, forming a small mass around the wound. When the fruit was cut, infected flesh appeared to be rotten, very soft or watery, and separated easily from the healthy tissue (Fig. 5B and H).

Symptoms on the fruit inoculated with *M. circinelloides* f. *lusitanicus* (X1785) appeared 2 weeks after the inoculation. Only a very small lesion was evident around the wound (about 5 to 7 mm in diameter) and became light or dark brown. Infrequently, very fine and white sporangiophores protruded through the wound, forming a flat thin layer over the wound. Often, no sporangiophores were observed around the wound. Decayed flesh inside the fruit became light yellowish, or no color change, and the decayed tissue became very soft and watery, and separated easily from the healthy tissue (Fig. 5C and I).

On the fruit inoculated with *M. circinelloides* f. *circinelloides* (X1899), no external symptoms were evident on the rind of the fruit during the course of experiments at both 5 and 20°C. No color change and no sporangiophores around the wound were observed. However, when the fruit was cut, the flesh under the wound became decayed and was light brown and very soft and watery, and separated easily from the healthy tissue (Fig. 5D and J).

Symptoms on the fruit inoculated with *M. hiemalis* (X2121) appeared 2 weeks after the inoculation. Only a small lesion (5 to 10 mm) developed around the wound, and the color of the lesion turned light or dark brown. Frequently, few and extremely thin sporangiophores protruded through the wound. Decayed flesh became light or dark brown and was very soft or watery, and separated easily from the healthy tissue (Fig. 5E and K).

Symptoms on the fruit inoculated with *M. mucedo* (X2142) were similar to those caused by *M. hiemalis*, but gray to dark masses of sporangiophores were often seen in the affected fruit flesh (Fig. 5F and L).

For all *Mucor* spp. tested at 20°C, the disease symptoms were similar to those observed at 5°C, except that the symptoms progressed faster. The five *Mucor* spp. were reisolated from decayed fruit that were inoculated with respective species.

On the cut fruit inoculated with the fungi, all *Mucor* spp. isolates tested were able to cause decay on mandarin fruit at both 5 and 20°C. No decay symptoms were observed on noninoculated control fruit. At 5°C, the two isolates of *M. piriformis*, X1816 and X1944, appeared to be the most virulent because the lesion sizes on the fruit inoculated with *M. piriformis* were the largest, followed by *M. mucedo* (X2142), *M. racemosus* f. *racemosus* (X1650), *M. hiemalis* (X2121), and *M. circinelloides* f. *lusitanicus* (X1785) (Table 6; Fig. 5). *M. circinelloides* f. *circinelloides* (X1899) resulted in no or very small

Fig. 4. Radial mycelial growth of the *Mucor* spp. on synthetic Mucor agar at various temperatures. Isolate names are followed by the initial letters of the species: P = *Mucor piriformis*, R = *M. racemosus* f. *racemosus*, C = *M. circinelloides* (X1785 = *M. circinelloides* f. *lusitanicus* and X1899 = *M. circinelloides* f. *circinelloides*), H = *M. hiemalis*, and M = *M. mucedo*.

Table 4. Radial mycelial growth of the five *Mucor* spp. on synthetic Mucor agar at 5 and 20°C in the dark

Temperature	Mycelial growth (mm/day) ^y								
	<i>Mucor piriformis</i>		<i>M. racemosus</i>		<i>M. circinelloides</i> ^z		<i>M. hiemalis</i>		<i>M. mucedo</i>
	X1816	X1944	X1650	X2081	X1785	X1899	X1946	X2121	X2142
5°C	8.7 a	8.2 a	2.8 bc	3.9 b	3.4 b	3.3 b	2.9 b	3.3 b	1.8 c
20°C	19.2 b	20.3 a	12.5 f	12.0 f	14.7 cd	14.1 de	13.5 e	15.0 c	10.4 g

^y Values followed by the same letter within the same row are not significantly different ($P = 0.05$) according to Tukey's honestly significant difference tests.

^z X1785, *M. circinelloides* f. *lusitanicus* and X1899, *M. circinelloides* f. *circinelloides*.

Table 5. Germination rate of sporangiospores of the five *Mucor* spp. on synthetic Mucor agar

Temperature ^z	Germination rate (%) ^w									
	<i>Mucor piriformis</i>		<i>M. racemosus</i>		<i>M. circinelloides</i> ^x			<i>M. hiemalis</i>		<i>M. mucedo</i> ^y
	X1816	X1944	X1650	X2081	X1785	X1899	X1935	X1946	X2121	X2142
5°C	88.0 a	83.2 a	20.6 c	18.7 c	0 e	0 e	0 e	7.1 d	44.3 b	0 e (19.2)
20°C	76.2 de	82.1 dc	96.1 a	94.2 ab	68.0 e	44.9 f	66.3 e	72.3 de	89.1 bc	0 g (44.8)

^w Values followed by the same letter within the same row are not significantly different ($P = 0.05$) according to Tukey's honestly significant difference tests.

^x X1785 and X1935, *M. circinelloides* f. *lusitanicus* and X1899, *M. circinelloides* f. *circinelloides*.

^y *M. mucedo* (X2142) did not germinate on synthetic Mucor agar at these two temperatures but germinated on orange juice agar, and the numbers in parenthesis are the germination rates on orange juice agar.

^z Incubated in the dark at 5°C for 48 h or 20°C for 14 h.

lesions 9 days after incubation at 5°C but was able to develop symptoms on the inoculated fruit after an additional 10-day incubation at 5°C. At 20°C, *M. piriformis* also appeared to be the most virulent species in comparison with the other species and *M. hiemalis* was least virulent, while *M. racemosus* f. *racemosus*, *M. circinelloides*, and *M. mucedo* did not differ significantly in lesion size (Table 6).

Discussion

This is the first report of Mucor rot in stored mandarin fruit in California. We documented that Mucor rot in mandarin in California was caused by five *Mucor* spp., consisting of *M. piriformis*, *M. circinelloides*, *M. racemosus*, *M. hiemalis*, and *M. mucedo*, and that *M. piriformis* was the dominant species, accounting for over 92% of the Mucor rot-decayed mandarin fruit sampled from three commercial citrus packinghouses, though the other four *Mucor* spp. were also pathogenic to mandarin fruit.

Previously, two *Mucor* spp., *M. racemosus* and *M. paronychius* (originally *M. paronychia*), were reported as citrus pathogens (Farr and Rossman 2015; Timmer and Menge 2000). *M. paronychius* was reported as a new *Mucor* sp. causing fingernail infection

among workers who squeezed cull orange fruit with bare hands (Sutherland-Campbell and Plunkett 1934). However, the establishment of *M. paronychius* as a species is considered questionable and this species was not studied by other authors (Farr et al. 1989; Walther et al. 2013). *M. paronychius* was neither included in the morphological studies on all accepted *Mucor* spp. by Schipper (1973, 1975, 1976, 1978) nor present in the phylogenetic analyses based on ITS or LSU D1/D2 region of the large ribosomal subunit sequencing in the order Mucorales by Walther et al. (2013). A large number of *Mucor* spp. names have been synonymized but we could not find such information about *M. paronychius*. Although Mucor rot in citrus has never been included in major widely used references for citrus diseases such as “Compendium of Citrus Diseases” (Timmer et al. 2000) or “A Color Atlas of Post-harvest Disease and Disorders of Fruits and Vegetables” (Snowdon 1990), association of *Mucor* spp. with decayed citrus fruit has recently been reported elsewhere. Bukar et al. (2009) conducted a pathogen survey in sweet orange (*Citrus sinensis*) in Nigeria and found that 25% of decayed fruit were associated with *Mucor* spp. Similarly, Akhtar et al. (2013) reported that 1.65% of decayed citrus collected from

Fig. 5. Symptoms of Mucor rot on mandarin fruit inoculated with representative isolates of the five *Mucor* spp. **A, G, and M,** *Mucor piriformis*; **B, H, and N,** *M. racemosus* f. *racemosus*; **C, I, and O,** *M. circinelloides* f. *lusitanicus*; **D, J, and P,** *M. circinelloides* f. *circinelloides*; **E, K, and Q,** *M. hiemalis*; and **F, L, and R,** *M. mucedo*. **A to F,** Fruit that were wounded, inoculated, and incubated at 5°C for 16 days in the dark; **G to L,** internal symptoms of the wound-inoculated fruit; and **M to R,** symptoms on the sliced fruit that were inoculated with the pathogen and incubated at 5°C for 15 days in the dark. Arrows indicate wound and inoculation sites.

Table 6. Lesion size (diameter in mm) on cut mandarin fruit inoculated with *Mucor* spp.

Temperature ^y	Lesion diameter (mm) ^w						
	<i>Mucor piriformis</i>		<i>M. racemosus</i>	<i>M. circinelloides</i> ^x		<i>M. hiemalis</i>	<i>M. mucedo</i>
	X1816	X1944	X1650	X1785	X1899	X2121	X2142
5°C	34.7 a	33.1 a	20.3 c	11.5 d	17.0 ^z	14.4 d	28.8 b
20°C	30.6 a	29.7 a	24.4 bc	22.1 c	20.6 c	16.7 d	20.6 c

^w Values followed by the same letter within the same row are not significantly different ($P = 0.05$) according to Tukey's honestly significant difference tests.

^x X1785, *M. circinelloides* f. *lusitanicus* and X1899, *M. circinelloides* f. *circinelloides*.

^y After incubation at 5°C for 9 days or 20°C for 2 days, diameter of the lesion on the cut surface of the fruit was measured.

^z Isolate X1899 developed no or a very small lesion after inoculation at 5°C for 9 days. The measurements were made after additional 10 days of incubation; thus, the data at 5°C were excluded from the statistical analysis.

cold-storage facilities in Pakistan were associated with *Mucor* spp. However, in both studies, *Mucor* spp. were not identified and no detailed descriptions of the disease symptoms were provided (Akhtar et al. 2013; Bukar et al. 2009). To the best of our knowledge, our study is the first report of *Mucor* rot in citrus fruit caused by *M. piriformis*, *M. circinelloides*, *M. hiemalis*, and *M. mucedo*.

Identification of *Mucor* spp. has traditionally been based on morphological characteristics and mating experiments, mainly studied by Schipper (1973, 1975, 1976, 1978). Recently, DNA barcoding markers have been successfully used for identification of species in the order Mucorales (Meyer and Gams 2003; Vitale et al. 2012; Walther et al. 2013). Walther et al. (2013) sequenced ITS regions of 178 species of the 227 currently accepted species in the order Mucorales and found that ITS sequencing proved to be a reliable tool to identify species in the order Mucorales because of its discriminative power. In the present study, all *Mucor* isolates were identified to the species level based on ITS sequencing, following the approach of Walther et al. (2013). In the present study, the identification based on the ITS sequencing was further confirmed by the morphological characteristics of the five *Mucor* spp. that were examined under the same culture growth conditions as those described by Schipper (1973, 1975, 1976, 1978) and compared with the descriptions of Schipper. We found that the descriptions by Schipper (1973, 1975, 1976, 1978) were accurate for the five *Mucor* spp. examined in this study, and suggest that such descriptions should continue to be used to aid speciation of *Mucor*. DNA sequencing provides a fast approach for identification of fungi. However, because the ITS region is variable between members in the order Mucorales, ITS sequences of some species may differ to such an extent that they could not be aligned confidently with their putative sibling species (Walther et al. 2013). Thus, in addition to ITS sequencing, examination of morphological characteristics is still necessary for accurate identification of *Mucor* spp.

We found that *M. piriformis* was the primary species responsible for *Mucor* rot in mandarin fruit. This finding is similar to *Mucor* rot in pome and stone fruit in California and the U.S. Pacific Northwest (Michailides and Spotts 1990b). In apple and pear, *M. piriformis* is the primary cause of *Mucor* rot, though *M. mucedo*, *M. racemosus*, and *M. strictus* have also been reported to cause decay (Spotts 2014). In stone fruit in California, *Mucor* spp. causing *Mucor* rot were recovered from orchard soils and identified as *M. piriformis*, *M. racemosus*, *M. circinelloides*, *M. hiemalis*, *M. plumbeus*, and *M. genevensis* (Michailides 1991). *M. piriformis* and other *Mucor* spp. are soilborne pathogens. They live as saprophytes in the soil. In the Central Valley of California, stone fruit and, to a lesser extent, apple are also grown. Citrus fields may be surrounded by stone or pome fruit orchards. Citrus trees may also be planted to fields previously planted with stone or pome fruit. The similarity in *Mucor* spp. compositions between citrus and stone fruit in California suggests that these *Mucor* spp. may be commonly present in orchard soils of these fruit crops in the region and that potential spread of inoculum of these fungi among orchards of different fruit crops could contribute to the build up of pathogen populations in the field because spores of *M. piriformis* on decayed fruit on the orchard floor can be spread by rodents, birds, and insects such as nitidulid beetles and vinegar flies (Michailides and Spotts 1986, 1990a; Spotts 2014).

Populations of sporangiospores of *M. piriformis* in pome fruit orchard soils fluctuate annually in response to soil moisture, temperature, and nutrient availability (Dobson et al. 1989; Guo et al. 1999; Spotts and Cervantes 1986). *M. piriformis* survives most successfully in cool, dry soils (Spotts and Cervantes 1986). In apple orchards in the Central Valley of California, populations of *M. piriformis* were highest during winter, declined during summer and fall, and increased again in winter and early spring of the following year (Guo et al. 1999). In the same region, mandarin fruit are harvested from late fall through winter to early spring, depending on cultivars (Kahn and Vidalakis 2014). The harvest time of mandarin fruit coincides with the occurrence of the highest populations of *M. piriformis* in apple orchard soils in the same region. If similar population dynamics of *M. piriformis* also occur in mandarin orchard soils, more inoculum

would be available during fruit harvest, leading to a high risk of *Mucor* rot infections. Research is needed to understand the survival and population dynamics of *M. piriformis* under the current cultural practices and environmental conditions in citrus orchards in California.

All five *Mucor* spp. tested in this study were able to actively grow at 5°C. The ability of these *Mucor* spp. to grow at the temperature (5°C) commonly used for storage of mandarin fruit supports the findings from pathogenicity tests that these fungi have the ability to cause decay on mandarin fruit in cold storage because the cold temperatures do not prevent the growth of these fungi. *M. piriformis* grew significantly faster at 5°C than the other four *Mucor* spp., supporting the results observed in the pathogenicity tests that *M. piriformis* is more virulent on mandarin fruit than the other *Mucor* spp. The high virulence of *M. piriformis* is also supported by its higher sporangiospore germination rates at 5°C than those of the other four *Mucor* spp. Similarly, studies to compare virulence of six *Mucor* spp. on stone fruit indicated that *M. piriformis* also is the most virulent species (Michailides 1991). Because *M. piriformis* is the most dominant and virulent *Mucor* spp. responsible for *Mucor* rot in mandarin fruit in California, control of *Mucor* rot in mandarin should target this species.

In pome fruit, *Mucor* rot-decayed fruit at the advanced stages release large quantities of juice containing sporangiospores of *M. piriformis*, which can spread from decayed fruit to healthy fruit, resulting in more infections during storage. Secondary spread of *Mucor* rot in cold storage is common in pear but not in apple (Spotts 2014). Secondary spread of *Mucor* rot from decayed fruit to sound fruit via ungerminated sporangiospores of *M. piriformis* also was observed on stone fruit (Michailides 1991). We did not determine whether and how *Mucor* rot could spread from infected fruit to sound fruit in cold storage; however, nesting of *Mucor* rot-decayed fruit has been observed in mandarin cold storage. Future research is needed to further understand infections of mandarin fruit by *M. piriformis* during cold storage. In addition, research also is needed to determine whether *Mucor* spp. affect other types of citrus fruit in California.

In this study, decayed fruit were collected from grower lots that were presorted and had been stored at 4.4 to 7.2°C for a period of time. Citrus postharvest fungicides were commonly applied at presorting operations to control decays caused by *P. digitatum*, *P. italicum*, and *G. citri-aurantii* (McKay et al. 2012; Smilanick and Mansour 2007; Smilanick et al. 2006, 2008). It is likely that the current decay-control programs may not be effective against *M. piriformis* because some existing citrus postharvest fungicides such as thiabendazole, pyrimethanil, and fludioxonil are known to be ineffective in controlling *M. piriformis* as observed on pome fruit (Spotts 2014). Nonetheless, research is needed to evaluate the effectiveness of sanitizers and fungicides that are commonly used at citrus presorting and packing operations in controlling spores of *Mucor* spp. and *Mucor* rot.

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