Role of *Monosporascus cannonballus* and Other Fungi in a Root Rot/Vine Decline Disease of Muskmelon

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ABSTRACT

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A serious disease of muskmelon (Cucumis melo) caused widespread losses in the Lower Rio Grande Valley of Texas in 1986 and has persisted through the 1990 crop season. Primary symptoms on the roots include extensive browning and necrosis of the taproot and lateral roots, vascular discoloration, and discrete brown to red cortical lesions. Secondary vine decline symptoms are characterized by a dieback of older crown leaves, which advances distally to younger leaves as the plants approach maturity. Four fungi (Fusarium solani, Monosporascus cannonballus, Macrophomina phaseolina, and Stagonospora sp.) were frequently isolated from the roots of diseased plants. Pythium spp., Cephalosporium sp., and F. oxysporum were also encountered but at relatively low frequencies. In greenhouse pathogenicity tests, M. cannonballus and M. phaseolina caused moderate to high levels of root rot and significantly reduced root weights of inoculated muskmelon plants. M. cannonballus also caused significant reductions in vine length, formed dark perithecia on the roots, and was reisolated from diseased plants. This is the first report of M. cannonballus in Texas and only the second from the United States.

Additional keywords: black spot root rot, cantaloupe, melon collapse

Muskmelon (Cucumis melo L.) is an important commercial crop in Texas. In 1990, approximately 10,000 ha were devoted to muskmelon, with most production coming from the Lower Rio Grande Valley. In 1986, a root rot disease of unknown etiology was observed in numerous Lower Rio Grande Valley muskmelon fields (5). The disease, also known as root rot/vine decline (RRVD),

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caused serious losses in 1986 and has continued to affect muskmelon production. The first noticeable aboveground symptom of RRVD is a gradual yellowing and dieback of the oldest crown leaves as the plants approach maturity. Foliar deterioration advances distally to younger leaves as the season progresses through the harvest period. Affected vines and plants decline and die prematurely. A high percentage of fruit from affected fields are unmarketable because of small size, low sugar content, or sun scald damage. These symptoms are strikingly similar to vine declines caused by Macrophomina phaseolina (Tassi) Goidanich, Didymella bryoniae (Auersw.) Rehm, Lasiodiplodia theobromae (Pat.) Griffon & Maubl., and Myrothecium roridum Tode:Fr. (2), except that vine lesions are associated with these diseases but are not characteristic of RRVD. Extensive areas of the taproot and some lateral roots darken, become necrotic, and may slough off as the plant is pulled from the ground. Discrete lesions, sometimes sunken and usually brown to red, often appear in the cortical tissues of affected roots. Brownish discoloration of the vascular tissues also occurs but rarely extends more than a few centimeters above the soil line.

Fusarium spp. have previously been implicated in the etiology of muskmelon root rot/vine decline (4,5). Several symptoms of RRVD resemble those of crown and foot rot of squash caused by F. solani (Mart.) Sacc. f. sp. cucurbitae W. C. Snyder & H. N. Hans. (16). Isolations from the roots of diseased plants in the Lower Rio Grande Valley consistently yielded F. solani (4,5), and frequently heavy sporulation of F. solani in some cortical lesions accounted for their unusual red coloration. In addition, the discovery of F. oxysporum Schlechtend.:Fr. f. sp. melonis Snyd. & Hans. in the Lower Rio Grande Valley coincided with the appearance of this vine decline disease (10). F. o. melonis, the causal agent of Fusarium wilt of muskmelon, causes some foliar symptoms similar to vine decline.

In a 2-yr study, numerous isolates of F. solani and F. oxysporum were ob-

tained from plants and soil in affected Lower Rio Grande Valley muskmelon fields and tested for pathogenicity (4). Most isolates were pathogenic to seeds and seedlings of the muskmelon cultivars Perlita and Magnum 45 in laboratory dish tests but failed to cause significant disease symptoms in greenhouse studies. Mixed inoculum consisting of a single isolate of F. solani combined with an isolate of F. oxysporum also gave negative results. An isolate of F. s. cucurbitae race 1 included in the screening tests as a positive control was pathogenic. Champaco concluded that the crown and root rot pathogen was not present in the Lower Rio Grande Valley and that F. solani, acting alone, was probably not responsible for the new "root rot-wilt" disease (4).

After these studies, the investigation was expanded to include other fungal genera associated with declining muskmelon plants. This report describes the symptomatology and presents pathogenicity data on Monosporascus cannonballus Pollack & Uecker and other rootassociated fungi.

MATERIALS AND METHODS

Specimen collection and observation. Four commercial farms with a history of root rot/vine decline were surveyed during May and June 1990 (Table 1). All four farms were located in a 50-km at the Tyner location, a single 3- to 4ha rectangular area was selected and sampled. Samples consisted of 20 symptomatic but still green plants excavated along a single diagonal through the sample area. Soil samples were collected along both diagonals with a 20-cm-long soil probe and bulked for each farm. In the laboratory, the crowns and root systems of each plant were washed and inspected for symptoms and signs of fungal infection. The pH and conductivity of the soil samples were determined from soil water extracts obtained by vacuum suction of soil water pastes (12).

Isolation and identification of fungi. Isolations were made from 10 plants per field, using tissues excised from the upper taproot, cortical lesions, and necrotic margins of small, lateral roots dying back from the tip. Excised tissues were surface-disinfested for 30-90 s in 0.5% NaOCl, rinsed once in sterile water, and incubated on one of four media-water

strip along the Rio Grande River in a two-county area that accounts for over half of the commercial muskmelon production in Texas (1). At each farm, with the exception of experimental plantings

Table 1. Site characteristics and frequency of Monosporascus cannonballus perithecia on roots of diseased muskmelon plants at four Lower Rio Grande Valley farms

Farm	Location	Soil pH	Soil conductivity ⁹	Cultivar	Plants with perithecia ^z (%)
SunTex	Rio Grande City	7.7	2.1	Laguna	95
Starrco	Rio Grande City	7.7	3.3	Laguna	95
Wiesehan	Edinburg	7.8	2.9	Durango	40
Tyner	Dona	7.8	2.7	Magnum 45	0

y Electrical conductivity of a soil water extract in millimohos/cm; 4.0 = saline soil conditions.

^{&#}x27;Twenty or more symptomatic plants from each farm were inspected for perithecia.

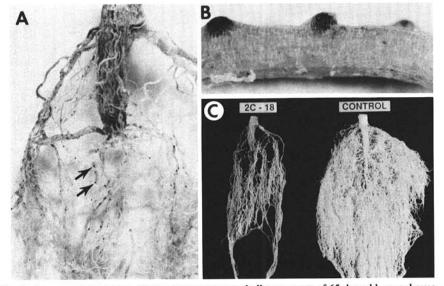


Fig. 1. Symptoms caused by Monosporascus cannonballus on roots of 65-day-old, greenhousegrown muskmelon plants. (A and B) Perithecia superficially embedded in 1- to 4-mm-diameter roots and (C) root damage caused by M. cannonballus (Texas isolate 2C-18 [= TX(Cm)90-25]) contrasted with an uninoculated control.

agar amended with 50 mg/ml of streptomycin sulfate (WA+); potato-dextrose agar acidified with 25% lactic acid (APDA); P₁₀ARP, selective for pythiaceous fungi (8); and Komada's medium, selective for F. oxysporum (9). After 5-7 days of incubation in darkness at 25 C, the isolation plates were examined at ×100 and colonies identified to genus when possible. Representative colonies of nonsporulating and unidentified fungi were transferred to V8 agar and PDA for further characterization.

Pathogenicity tests. Forty-six fungal isolates were screened for pathogenicity in simultaneous laboratory and greenhouse tests. Both experiments used the same inoculum preparation, muskmelon cultivar (Magnum 45), and fungal isolates. With the exception of Pythium spp., which were grown on V8 agar, all isolates were grown for 9-10 days on PDA in 9-cm-diameter plastic petri dishes. Inoculum consisted of a mycelial suspension prepared by blending a petri dish culture of each isolate in 100 ml of sterile distilled water for 40 s in an Eberbach microblender. Thiram-treated seeds from a commercial seed lot of Magnum 45 muskmelon were washed in a 0.5% solution of Sparkleen detergent for 5 min and rinsed three times with distilled water before use in the greenhouse tests. After this preliminary treatment, seeds to be used in the laboratory seedling assay were surface-disinfested in 0.25% NaOCl for 2 min, rinsed three times with sterile distilled water, and pregerminated under sterile conditions on a water-moistened pad. One milliliter of inoculum suspension was spread across the surface of 100 ml of solidified 1% water agar in a 100 × 80 mm (diameter × height) petri dish. Three pregerminated seeds were placed in each dish, and two dishes were inoculated with each isolate. Three dishes with added sterile water served as controls. The seeds were incubated at 23-26 C beneath a north laboratory window for 14 days when the seedlings were evaluated for symptom development.

In the greenhouse tests, 50 ml of inoculum suspension was poured into 15cm-diameter plastic pots filled to within 5-7 cm of the top with a 4:1 (v/v) mixture of steamed sand and pearlite and stirred into the top 3-4 cm of the potting mix. An additional 4-5 cm of clean mix was added to top off the pots and three seeds were planted in each pot. Two pots were infested with each isolate and six pots received a sterile suspension of PDA in water to serve as controls. After 12 days, the plants were thinned to one per pot and fertilized weekly with 200 ml of 0.1% Peters 20:20:20. Diurnal temperatures in the greenhouse ranged from 25 to 32 C. The experiment was concluded after 65 days when the plant root systems were rated for disease on a scale of 0 (healthy) to 4 (extensive browning or lesion development on the taproot and several lateral

roots). Isolations were made from the roots of plants exposed to *M. cannon-ballus*, *Pythium* sp., and *Stagonospora* sp. Root dry weights and lengths of the primary vines were recorded.

Disease severity index data was analyzed by the Kruskal-Wallis nonparametric test followed by a paired multiple comparison procedure (6). Measurement data, e.g., vine length and root dry weights, were subjected to one-way analysis of variance (GLM procedure) and Duncan's multiple range test on SAS (SAS Institute, Inc., Cary, NC).

RESULTS

Specimen collection and observation. Perithecia of M. cannonballus were observed on the roots of field-grown plants showing RRVD symptoms at three of the farm locations (Fig. 1A). The frequency of occurrence of perithecia on these plants ranged from 40 to 95% (Table 1). Small necrotic roots 1-3 mm in diameter often supported large numbers of perithecia, which were visible to the naked eye as small black bulges in the root cortex. Infected roots were typically fragile and shrunken but nearly normal in color, i.e., off-white to light brown. Microscopically, mature perithecia appeared as dark, roughly circular bodies 230-350 µm in diameter (Fig. 2A and B) filled with numerous clavate asci, each containing a single, dark, spherical ascospore (Fig. 2C and D) characteris-

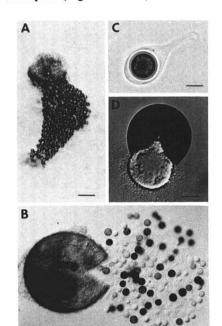


Fig. 2. Monosporascus cannonballus. (A) Group of ascospores discharged from a perithecium formed on potato-dextrose agar. Scale bar = 150 μ m. (B) Squash mount of a perithecium formed on host root tissue, showing mature (dark) and immature (light) ascospores. Scale bar = 75 μ m. (C) Typical ascus with a single, dark ascospore. Scale bar = 20 μ m. (D) Squash mount of a single ascospore showing cytoplasmic contents. Scale bar = 10 μ m.

tic of the species (9). The identity of *M. cannonballus* was confirmed by F. A. Uecker, USDA-ARS, Beltsville, MD (personal communication), and a representative isolate has been sent to the American Type Culture Collection (ATCC 76452). The soil at all four farms was alkaline with conductivity readings indicating moderately elevated soil salt levels (Table 1).

Isolation and identification of fungi. Four genera of fungi were isolated with regularity from the root systems of the 40 mature plants collected from the four farms (Table 2). Two species, F. solani and M. cannonballus, accounted for over half of the 357 isolates obtained. M. phaseolina was the third most frequently isolated fungus. The fourth was a slow-growing fungus that produced dark stromatic colonies on APDA. Representative isolates were preserved on their original isolation plates and also transferred to V8 agar and PDA. After 2-3 wk of incubation, several of the isolates

produced pycnidia on the tissue in the WA⁺ isolation plates and near the transfer plug on V8 agar. These isolates were identified as *Stagonospora* sp.

The numbers and types of fungi isolated varied with farm location and plant tissue type. Most Cephalosporium isolates were obtained from the Starrco farm, whereas Pythium spp. were not found at this site (Table 2). M. cannonballus was isolated at high frequency from three of the farms but was not isolated from the Tyner site. However, the majority of isolates of Stagonospora were obtained from the Tyner farm. F. solani was consistently isolated at high frequency from all four locations. Relatively few fungi were isolated from internal taproot tissue near the soil line, although most isolates of Cephalosporium and F. oxysporum came from this tissue. Pythium spp. were not isolated from upper taproot tissues. M. phaseolina and Pythium spp. were more frequently associated with cortical lesions, whereas

Table 2. Identities and numbers of fungi isolated from roots of diseased muskmelon plants collected from four farms in the Lower Rio Grande Valley

	Number of fungi isolated from each farmy				All
Fungus	SunTex	Starrco	Wiesehan	Tyner	farms
Fusarium solani	35	35	27	33	130
Monosporascus cannonballus	20	29	21	0	70
Macrophomina phaseolina	12	13	9	8	42
Stagonospora sp.	8	3	8	21	40
Pythium sp.	8	0	6	8	22
Cephalosporium sp.	0	6	1	2	9
F. oxysporum	3	1	4	0	8
Miscellaneous fungi ²	18	7	10	11	46

y Each column represents the number of isolates obtained from the taproot, secondary, and tertiary roots of 10 diseased plants. One hundred twenty-six, 144, 128, and 128 pieces of tissue were plated out from SunTex, Starrco, Wiesehan, and Tyner farm specimens, respectively.

Numerous fungi were isolated at low frequencies, e.g., species of Alternaria, Curvularia, Fusarium (other than F. solani and F. oxysporum), Rhizoctonia, and members of the Helminthosporium complex.

Table 3. Pathogenicity of fungi to muskmelon plants in laboratory and greenhouse screening tests

	Isolates ^u (no.)	Laboratory test* Diseased plants (%)	Greenhouse test*		
Fungus			Root disease index x	Root dry weight (g)	Vine length (cm)
Monosporascus cannonballus	8	60	2.8 b ^y	0.79 c ^z	108 c ^z
Macrophomina phaseolina	6	72	1.2 b	0.83 c	168 a
Pythium spp.	6	33	0.3 a	1.17 a	144 b
Stagonospora sp.	8	15	0.2 a	0.95 bc	153 ab
Cephalosporium sp.	4	29	0.1 a	0.96 bc	158 ab
Fusarium solani	8	13	0.1 a	1.08 ab	152 ab
F. oxysporum	6	19	0.0 a	0.95 bc	156 ab
Control		0	0.2 a	1.12 ab	165 ab

"All isolates were screened in parallel laboratory and greenhouse tests.

Six pregerminated seeds were exposed to each isolate in petri dishes in the laboratory; data are percent seedlings showing disease symptoms after 14 days.

"Two plants, each in a separate pot, were inoculated with each isolate in the greenhouse; each figure represents the pooled average for all isolates of a given species or genus.

*Root disease was evaluated on a scale from 0 (healthy) to 4 (extensive areas of taproot turning brown or covered by lesions; several secondary roots browning or with multiple lesions).

y Population distributions (represented by average root disease indices in this column) followed by the same letter are not significantly different at the P=0.05 level according to a multiple comparison procedure following a Kruskal-Wallis nonparametric test.

Numbers in a column followed by the same letter are not significantly different at the P = 0.05 level according to Duncan's multiple range test.

nearly equal numbers of F. solani, M. cannonballus, and Stagonospora sp. were obtained from cortical lesions and roots that were dying back from the tip.

Pathogenicity tests. The laboratory and greenhouse screening tests demonstrated similar trends in pathogenicity among the seven groups of fungi tested (Table 3). M. phaseolina and M. cannonballus were aggressive pathogens in the laboratory seedling tests, causing disease symptoms in 72 and 60% of the inoculated plants, respectively (Table 3). Both fungi caused a generalized browning of the taproot, followed by collapse of the hypocotyl. Two Pythium isolates rapidly attacked the pregerminated seeds and prevented further growth. The remaining fungi affected fewer of the inoculated plants and produced generally milder symptoms. For example, some isolates of F. solani, F. oxysporum, and Cephalosporium induced a slight yellowing of the taproot, and several Stagonospora isolates caused a conspicuous pinkish coloration in infected roots and colonized seed coats.

Under greenhouse conditions, most isolates of M. cannonballus and M. phaseolina damaged the roots of inoculated muskmelon plants. When data were pooled for each group of isolates, M. cannonballus and M. phaseolina were the only fungi that significantly reduced root dry weights (Table 3). Both fungi also caused significant levels of root disease. However, M. cannonballus, with a root disease index (RDI) of 2.8, caused considerably more root deterioration than M. phaseolina (RDI = 1.2). M. cannonballus also caused dramatic stunting of the plants, reducing primary vine lengths by 35% compared with those of the uninoculated controls (Table 3). None of the other fungi, including M. phaseolina, caused significant reductions in vine length.

A total of eight isolates of M. cannonballus were tested. Four were aggressive pathogens in the greenhouse tests and produced corresponding high disease incidences in the laboratory seedling tests (Table 4). In the greenhouse tests, these four isolates produced an average root disease rating of 3.9 on a scale of 4.0 and caused reductions in root dry weight and vine length of 57 and 54%, respectively (Table 4 and Fig. 1C). Young perithecia of M. cannonballus were observed on the roots (Fig. 1A and B), and the fungus was reisolated from 75% of the plants inoculated with these isolates. The remaining four isolates of M. cannonballus displayed reduced pathogenicity, produced few perithecia, and were reisolated from inoculated plants at lower frequencies.

The other fungi produced little or no damage in the greenhouse tests (Table 3). However, a conspicuous reddening of one or more small lateral roots was noted in some plants exposed to *Stagnospora* sp. and the fungus was infrequently reisolated from these areas. *Pythium* spp. were also occasionally recovered from their respective inoculated plants. None of the test fungi were isolated from plants serving as negative controls.

DISCUSSION

The survey of infected plants at four Lower Rio Grande Valley locations consistently associated vine decline symptoms with diseased root systems. Most fungi isolated from these diseased roots belonged to six genera. Three of these genera, Monosporascus, Stagonospora, and Cephalosporium, have not been reported on muskmelon in the Lower Rio Grande Valley. M. cannonballus is of particular interest because of its high frequency of occurrence on the roots of diseased plants and its demonstrated pathogenicity in laboratory and greenhouse

Table 4. Pathogencity of eight Texas isolates of *Monosporascus cannonballus* to muskmelon seedlings in laboratory and greenhouse tests

	Laboratory test*	Greenhouse test*				
Isolate	Diseased plants (%)	Root disease index*	Root dry weight (g)	Vine length (cm)		
TX(Cm)90-25	100	4.0 d ^y	0.38 e ^z	73 de ^z		
TX(Cm)90-24	83	4.0 d	0.48 cd	51 e		
TX(Cm)90-30	67	3.8 d	0.61 cd	89 cd		
TX(Cm)90-23	83	3.8 d	0.73 bc	90 cd		
TX(Cm)90-29	50	3.0 c	0.94 ab	136 ab		
TX(Cm)90-26	67	2.8 bc	1.01 ab	148 ab		
TX(Cm)90-27	17	1.3 a	1.08 a	159 a		
TX(Cm)90-28	17	0.0 a	1.10 a	119 bc		
Control	0	0.3 a	1.27 a	163 a		

Six pregerminated seeds were exposed to each isolate in petri deep dishes in the laboratory; data gives percent seedlings showing disease symptoms after 14 days.

screening tests. Pathogenicity was confirmed in a second greenhouse test in which TX(Cm)90-26, an M. cannonballus isolate showing intermediate aggressiveness in the initial screening tests, caused significant (P = 0.05) reductions in root weight and vine length of muskmelon plants when deployed as a sandcornmeal inoculum (data not presented). That there are isolates of M. cannonballus that vary in aggressiveness and virulence poses problems in assessing field infestation levels. It is not known what percentage of the M. cannonballus population in a given field is pathogenic or nonpathogenic.

The only published report of M. cannonballus in the United States came from Arizona in 1970 (17). The authors reported the isolation of an unknown fungus "typified by numerous scattered, small, round, black bodies" from rotted secondary roots of cantaloupe plants. The characterization of this fungus by Pollack and Uecker (13) in 1974 resulted in the erection of the genus Monosporascus. An Arizona isolate was designated as the holotype. M. cannonballus has since been reported from Japan, where it causes a root rot of melon (C. melo) growing in plastic tunnels or under plastic mulch (18), and from India (7). A recent unpublished report suggests that M. cannonballus is present in California (M. E. Stanghellini, University of Arizona, personal communication). A related species, M. eutypoides (Petrak) von Arx, has been associated with "collapse disease" of melon plants in Israel (14) and has been isolated from darkened bases of Triticum stems in Libya (7).

Most Monosporascus collections have come from relatively hot, dry areas of the world (7,14,17). Soils in these areas tend to be alkaline and may have problems attributable to accumulated salts. The Lower Rio Grande Valley of south Texas is similar to these areas in climatic and edaphic characteristics. During the April-June harvest period for muskmelons, soil temperatures at a depth of 10 cm range from 24 to 31 C in the Lower Rio Grande Valley. These temperatures are ideal for a genus with a subterranean growth habit and temperature optimum between 25 and 35 C (7,14,18). During this study, Monosporascus was discovered on three farms on which the coarsetextured soils were similar in pH (7.7-7.8) and soil salt content (moderately elevated), suggesting that the fungus may prefer or tolerate alkaline and saline conditions.

M. cannonballus was only one of several fungi that occurred on roots of diseased muskmelon plants in the Lower Rio Grande Valley. F. solani was ubiquitous and was isolated at high frequency from roots of plants with RRVD symptoms. However, selected Lower Rio Grande Valley isolates of F. solani were nonpathogenic in greenhouse screening

[&]quot;Two plants, each in a separate pot, were inoculated with each isolate in the greenhouse.

^x Root disease evaluated on a scale from 0 (healthy) to 4 (extensive areas of taproot and one or more secondary roots turning brown).

^y Population distributions (represented by average root disease indices in this column) followed by the same letter are not significantly different at the P = 0.05 level by a multiple comparison procedure following a Kruskal-Wallis nonparametric test.

² Numbers in a column followed by the same letter are not significantly different at the P = 0.05 level according to Duncan's multiple range test.

tests during this and a previous study (4). In contrast, isolates of M. phaseolina were pathogenic in greenhouse tests. These results corroborate work by Carter (3) and Reuveni et al (15), who described M. phaseolina as a causal agent of muskmelon vine decline in the Lower Rio Grande Valley and Israel, respectively. Although M. cannonballus was not observed or isolated from Tyner farm specimens, other fungi were associated with declining plants at this location. Both pathogenic isolates of Pythium originated from Tyner farm. Pythium spp. have been implicated as causal agents of "sudden wilt," a rapid decline disease of muskmelon in California (11). In addition, a Stagonospora sp. was obtained at relatively high frequencies from Tyner farm. Laboratory and greenhouse experiments failed to demonstrate significant levels of pathogenicity for the eight isolates of Stagonospora tested; however, two were reisolated from small, reddish necrotic lateral roots occasionally observed on plants from the greenhouse test. This is the first report of Stagonospora sp. on C. melo in the United States.

The spectrum of fungi obtained from the Starrco field was unusual. A Cephalosporium sp. was obtained at relatively high frequency, but Pythium spp. were not isolated. Plants in this field were grown under black plastic mulch and irrigated by buried drip lines; those at the other survey sites were grown on bare soil with furrow irrigation. The practice of plastic mulching is being adopted in the Lower Rio Grande Valley for weed control and to provide muskmelon seedlings with warmer soil conditions early in the growing season. Its effect on soil

microflora, especially plant pathogenic microorganisms, merits further study.

M. cannonballus is the current focus of investigation because it accounts for much of the new vine decline disease observed in the Lower Rio Grande Valley. Monosporascus root rot/vine decline can be distinguished from other known vine decline diseases of muskmelon by the presence of black perithecia on the roots and the absence of vascular discoloration or lesions in aboveground plant parts. The perithecia embedded in infected roots are striking and diagnostic and are unlikely to be confused with the smaller, irregular sclerotia of M. phaseolina. Because of this, the disease has been referred to as "black spot root rot" by Japanese researchers (18). Although this name is appropriate in many cases, perithecia are not found on the roots of recently infected plants and may not be as numerous or visible on new hosts that may be discovered in the future. Therefore, we propose the name Monosporascus root rot/vine decline for this new disease of muskmelon.

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