Occurrence and Pathogenicity of Fungi Associated with Melon Root Rot and Vine Decline in California

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ABSTRACT

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The occurrence of fungi associated with root rot and vine decline of melon (*Cucumis melo*) in commercial fields in California was surveyed over 3 years. The fungi most frequently isolated from discolored vascular tissue or root rot were *Acremonium cucurbitacearum*, *Rhizopycnis vagum*, *Monosporascus cannonballus*, *Fusarium solani*, *Macrophomina phaseolina*, *Pythium* spp., and *Verticillium dahliae*. The frequency of isolation of the various fungi varied with root symptomology. *Pythium* spp., and *M. phaseolina* were frequently associated with a wet, brownish root rot, while *A. cucurbitacearum*, *R. vagum*, and *Rhizoctonia solani* were generally associated with a dry, corky root rot. Presence of *Monosporascus cannonballus* was associated both with a wet, brownish rot as well as with discrete, reddish, corky lesions. The frequency of isolation of a given pathogen varied with geographic location, with *M. cannonballus* present only in the southern production areas, while *A. cucurbitacearum* and *Rhizopycnis vagum* were most common in the northern production areas. In pathogenicity tests in field microplots, *M. cannonballus* caused vine collapse and severe root rot of cantaloupe, reducing root length density by 93%. California isolates of *R. vagum* and *A. cucurbitacearum*, although only weakly pathogenic in field microplots, caused root discoloration and reduced vine growth in greenhouse tests. Reduction in dry weight of greenhouse-grown cantaloupe was 40, 23, and 39% for *R. vagum*, *A. cucurbitacearum*, and *M. cannonballus*, respectively.

Approximately 37,500 ha of cantaloupe and honeydew (*Cucumis melo* var. *reticulatus* and var. *inodorus*) were planted in California in 1999, representing 68% of the U.S. national production (7). A root rot and associated vine decline have been continuing problems on many types of melons, including cantaloupe, honeydew, various mixed melons, and watermelon (*Citrullus lanatus*). Aboveground symptoms include yellowing and death of the crown leaves. Sometimes, only scant or no evidence of root rot is apparent but, in most cases, root symptoms include a rot of secondary and feeder roots and reddish or corky lesions on the taproot. Although root vascular tissue is sometimes discolored, this rarely extends into the stem. Vine collapse typically occurs just prior to harvest, resulting in premature fruit ripening, low sugar accumulation, and exposure of fruit to the sun.

A number of fungi have been reported as causal agents of melon collapse worldwide, but descriptions of vine and root symptoms have often been overlapping and the cause of vine collapse in many cases is unclear. In 1970, Troutman and Matejka (25) concluded that *Rhizoctonia solani*, *Verticillium albo-atrum*, and an unidentified fungus which was later described as a genus et species novus, *Monosporascus cannonballus* (20), were the primary fungi associated with cantaloupe root rots in Arizona. In Texas, a root rot-vine decline of muskmelon was first reported in 1988 (8) and was subsequently attributed to *M. cannonballus* (15). *M. cannonballus* has also been reported from California (5). A sudden wilt of melons in California has been attributed to *Pythium ultimum*, *P. aphanidermatum*, and *P. myriotylum* (3,12,13). In Spain, *Acremonium cucurbitacearum* was the most commonly isolated fungus from muskmelons suffering from "sudden death" on the eastern Mediterranean coast (10). *A. cucurbitacearum* has also been associated with vine decline in parts of the Central Valley of California (5). A *Stagonospora* sp.-like fungus has been implicated in vine decline in the Rio Grande Valley of Texas (16) and was recently described as a genus et species novus, *Rhizopycnis vagum* (9). A corky root

rot of melons in France is caused by *Pyrenochaeta lycopersici* (22).

The objective of this work was to survey melon fields in California for the occurrence of root-infecting fungi and to test their pathogenicities under field and greenhouse conditions. In addition, symptomology associated with root colonization by these fungi is described.

MATERIAL AND METHODS

Disease survey. To determine the occurrence of fungi associated with melon roots, samples of cantaloupe, honeydew, and occasionally mixed melon and watermelon roots were obtained over a 3-year period (1995 to 1997) from fields in 11 counties in California. In 1995, samples were obtained from 30 fields where plants suffered from wilt, premature vine decline, or root rot. In 1996, samples were obtained from symptomatic plants in 27 fields. Soil samples were also obtained from 23 fields in 1996. Samples were composites of 15 to 30 cores taken from the surface soil to 15 cm deep. In 1997, roots from 24 fields were sampled.

To remove soil, the root systems were washed in 1 liter of 1% sodium metaphosphate on a shaker for 15 to 20 min. After symptoms were noted, isolations were made from cortex and vasculature of the tap root, secondary and tertiary roots, the hypocotyl, and stem vascular tissue of four to six plants from each field, regardless of whether the specific tissue was symptomatic. The pieces of root tissue were surface sterilized in 0.525% sodium hypochlorite for 2 min and plated on 1.5% water agar amended with 100 ppm streptomycin sulfate (SWA). When symptoms were typical of pythiaceus fungi (a wet, brownish decay; 12), symptomatic tissue pieces also were plated on a selective medium consisting of corn meal agar (Difco Laboratories, Detroit) amended with 10 ppm pimarin, 250 ppm ampicillin, 10 ppm rifampicin, and 25 ppm pentachloronitrobenzene. Some tissue pieces were placed in a moist chamber for 7 to 10 days to allow development of pycnidia or perithecia.

After the plates were incubated at room temperature for 3 to 5 days, hyphal tips or spores of various fungi were transferred to

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potato dextrose agar (PDA, Difco Laboratories) or 20% V8 agar for identification. *Fusarium* spp. were identified based on morphological characteristics on carnation leaf agar (17). The frequency of isolation of each fungus from tissue types and field locations was recorded.

Ascospores of *M. cannonballus* were extracted from the field soil samples by a method adapted from Stanghellini and Rasmussen (24). After the entire sample was sifted through a 4-mm screen, four 20-g subsamples were each added to 200 ml of water and stirred for 5 min using a magnetic stirrer. Each subsample was then

washed through nested 75- and 38- μ m sieves for 5 min. The material retained by the 75- μ m sieve was discarded while that retained on the 38- μ m sieve was centrifuged at 4,000 rpm ($2,500 \times g$) for 2 min in 40 ml of 75% sucrose. The supernatant was decanted onto the 38- μ m sieve and rinsed with water. The material retained on the sieve was stored in a small quantity of water at 4°C until it was analyzed. In this way, material of roughly the same size and density as *M. cannonballus* ascospores was separated from most of the other soil and organic matter. Spores in the water were enumerated under a stereomicroscope at a

magnification of 40 $\times$ in a petri dish imprinted with a roughly 1-cm² grid. Spores were counted if they were shiny black, entirely smooth, and 35 to 50 μ m in diameter. The four subsample values from each composite sample were averaged. These were composite samples; therefore, the variation between subsamples was due to the extraction method and not to variation across the field. Hence, statistical comparisons of spore counts between fields could not be made.

Inoculum production. All isolates used in pathogenicity tests were obtained from symptomatic melon roots in California.

Fig. 1. Symptoms associated with (A and B) *Acremonium cucurbitacearum* and (C) *Rhizopycnis vagum* in diseased commercial fields. Symptoms caused by *A. cucurbitacearum* on cantaloupe inoculated in (D) field microplots and (E) the greenhouse. (F) Symptoms caused by *Rhizopycnis vagum* on cantaloupe inoculated in the greenhouse.

Single-spore isolates of *A. cucurbitacearum* and *Fusarium* spp. were obtained prior to use in pathogenicity tests, while hyphal tip isolates of *R. vagum* and *M. cannonballus* were used. Inoculum for field and greenhouse trials was grown on a mixture of washed quartz sand and ground oat hulls (Quaker Oats, Chicago), combined at a rate of 45 g of oat hulls to 500 cm³ of sand. In 1-liter flasks, 100 ml of water was combined with 500 cm³ of this medium and autoclaved twice for 60 min with a 1-day interval. The medium was inoculated with two to three 1-cm² pieces of colonized agar cut from a PDA culture. The flasks were incubated at room temperature under 12 h of fluorescent light per day. After the hyphae had grown out 2 to 3 cm, the flasks were shaken to distribute the mycelium. Colonization of the substrate was completed in 5 to 7 weeks. In one field trial (1995), inoculum of *A. cucurbitacearum* and *M. cannonballus* was grown in a similar manner on sterilized millet. Microsclerotial inoculum of *V. dahliae* was prepared by growing the fungus in liquid culture which was then spread on sheets of sterile cellophane overlying Puhalla's minimal agar medium (21) in trays (33 by 53 cm). After 2 to 3 weeks, the cellophane was removed, homogenized in a blender with water, and combined with sand.

The CFU concentration of the colonized sand and oat-hull substrate was determined by serial dilution. Once the flasks of inoculum had been bulked, two or three 1-gm samples each were added to 99 ml of 0.2% water agar (WA) and mixed with a magnetic stirrer for 10 min to prepare a 100-fold dilution. The 10³ to 10⁶ dilutions were prepared by adding 1 ml of the previous dilution to 9 ml of 0.2% WA. Three 1-ml subsamples of each dilution were plated on PDA and incubated at room temperature for 3 days. The number of colonies per plate was multiplied by the dilution factor to obtain the CFU/g of inoculum on a moist-weight basis. The counts obtained from the three subsample plates, the various dilutions, and the two or three samples were averaged. CFU concentrations utilized in pathogenicity testing were determined by preliminary experiments or adapted from reports in the literature (6).

Field pathogenicity tests. In 1995, 1996, and 1997, field microplots were created by digging a trench into which plastic cylinders (75 cm in diameter) were placed; soil was filled back in around and within the cylinders so the bottom of the plots was roughly 46 cm below the soil level. Newly constructed microplots were used each year. In some treatments, 43 ml of metam-sodium was added to water in each of the plots at a rate equivalent to 935 liters/ha of metam-sodium. After at least 3 weeks, inoculum was mixed into the upper 15 cm of the treated plots, while the control plots remained untreated. Plots were direct seeded with the cantaloupe cv. Mag-

num .45 and thinned to four plants/plot. All plots were covered with finely meshed nylon row covers to protect the young plants from virus vectors until flowering and vining. All plots were fertilized once at thinning with ammonium sulfate at a rate of 168 kg of N/ha and watered by drip-irrigation or hand watering as needed. The crop was grown to horticultural maturity (80 to 90 days after seeding).

In 1995, two experiments were conducted in the field microplots. In the first experiment, planted in June, the soil was not treated with metam-sodium. Plots were inoculated with *M. cannonballus* (3.6 kg of colonized millet/plot), *A. cucurbitacearum* (3.6 kg of colonized millet/plot), or nothing. In the second experiment, planted in July, all plots received the metam-sodium treatment and were inoculated as above with *M. cannonballus*, *A. cucurbitacearum*, *V. dahliae*, or non-colonized millet. No measurements of CFU concentrations of the inoculum were made. In both experiments, each treatment was replicated four times in a randomized complete block design. In an experiment planted in July 1996, plots were inoculated with *A. cucurbitacearum* (1.5 kg of colonized sand-oat mixture/plot, 17,000 CFU/g soil), *M. cannonballus* (0.75 kg of colonized sand-oat mixture/plot, unknown CFU concentration), *F. solani* (1 kg of colonized sand-oat mixture/plot, 5,500 CFU/g soil), *R. vagum* (1.3 kg of colonized sand-oat mixture/plot, unknown CFU concentration), or nothing. Each inoculation treatment included three plots that had been treated with metam-sodium prior to planting and three that had not. In a second experiment, also planted in July, plots were treated with metam-sodium and then inoculated (at same rates as above) with *A. cucurbitacearum*, *M. cannonballus*, or nothing, and all plots were watered with about half the frequency of plots in the first experiment. In all experiments, each treatment was replicated three times in a randomized complete block design.

In 1997, plots were inoculated with *M. cannonballus* (2.4 kg/plot, 400 CFU/g soil), *A. cucurbitacearum* (2.8 kg/plot, 3,000 CFU/g soil), *R. vagum* (3.5 kg/plot, 6,500 CFU/g soil), or nothing. All plots were treated with metam-sodium prior to planting in June. All treatments were replicated three times in a completely randomized design.

In all experiments, plants were harvested when the fruit was mature. Roots and lower stems were gently removed from the plots, washed in 1% sodium metaphosphate, photographed, and rated for disease severity on the following scale: 0 = no symptoms; 1 = few lesions (covering <10% of root), secondary root rot slight; 2 = rot of secondary roots or lesions covering approximately 25% of the root; 3 = lesions covering at least 50% of the root and dead secondary roots; and 4 = general root rot, most of the root affected. Representative

symptomatic tissues were surface-sterilized for 2 min in 0.525% sodium hypochlorite and plated on SWA.

In 1997, estimation of root density in each treatment at the end of the season was conducted by a method adapted from Ward et al. (26). A core (2 cm in diameter) from the surface to 20 cm in depth was collected about 3 cm from the crown of each of the four plants in each plot. The four cores from each plot were combined and added to 300 ml of water. After the water was swirled and large soil particles were allowed to settle, the water was decanted through a 250- μ m sieve. This process of adding water, allowing large soil particles to settle, and decanting was repeated twice. In this way, the organic matter (larger than 250 μ m) in the sample was separated from most of the soil particles. The organic matter was then added to water several millimeters deep in a clear plastic tray (13 by 18 cm) overlaying a 1-cm grid. Intersections of the grid lines with live roots visible to the naked eye were counted. Estimation of root length was made using the equation of Newman (18). To assess the accuracy of this estimation, a thread (55 cm long) was spread on the tray and its intersections with the grid lines counted. Whether the thread was entire or cut into sections (1 to 2 cm long), the equation provided an approximation of its length within about $\pm 0.9\%$.

Greenhouse pathogenicity tests. The pathogenicities of *R. vagum*, *A. cucurbitacearum*, and *M. cannonballus* were evaluated in separate experiments in the greenhouse. The fungi were tested on watermelon (*C. lanatus* cv. Crimson Sweet), honeydew (*C. melo* var. *inodorus* cv. Green Flesh), squash (*Cucurbita pepo* cv. Super Set), and cantaloupe (*C. melo* var. *reticulatus* cvs. Magnum .45 [F1 hybrid] and Top Net [open pollinated]). The experiments were conducted in 3.8-liter pots filled with potting mix (40% washed sand, 20% nitrified redwood, 20% sphagnum peat moss, 20% pumice rock, and a trace of lime) and sterilized Yolo sandy loam combined 50:50 on a volumetric basis (2.5 kg of mixture/pot). Inoculum was mixed with the medium at 5,000 CFU/g of soil for *R. vagum*, 20,000 CFU/g for *A. cucurbitacearum*, and 30 CFU/g for *M. cannonballus*. The plants were direct seeded, thinned to two plants per pot, fertilized once with 4 g of a slow-release fertilizer (Apex 21-5-6 plus micronutrients, J. R. Simplot Co., Boise, ID), and watered as needed. In all greenhouse experiments, the temperature ranged from a minimum of 20°C to a maximum of 27°C (winter) or 33°C (summer). Plants were harvested at 40 days (*A. cucurbitacearum* experiments) or 50 days (*R. vagum* and *M. cannonballus* experiments) after seeding. Each experiment was repeated once.

At harvest, the length of the main shoots was measured and roots were recovered by

gentle washing of the root ball from the pot. Roots were rated for disease severity based on the following scale: 0 = no symptoms, 1 = slight or limited area of discoloration, 2 = general discoloration (one or two lesions), 3 = general discoloration (rot of some tissues), and 4 = rot of entire tap and feeder roots. A small piece of representative root tissue was plated on SWA to isolate fungi. Weights were measured after the tissue was dried in an oven at 50°C for 2 to 3 days.

Statistical analyses. Analysis of variance was used to evaluate differences in shoot length, dry weight, and disease severity in greenhouse experiments and differences in root density and disease severity in microplot experiments. Measurements were transformed to their natural log equivalents, when necessary, to eliminate heteroscedasticity. Mean separations were determined using Fisher's least significant difference ($P = 0.05$). Statistical computations were performed using the PROC GLM procedure in SAS (version 6; SAS Institute, Cary, NC).

RESULTS

Disease survey. *A. cucurbitacearum*, which was recovered from plants in 32% of the surveyed fields, and *R. vagum* (31%) were the fungi most commonly isolated from symptomatic melon roots over a 3-year period. Other commonly isolated fungi included *Pythium* spp. (23%), *Macrophomina phaseolina* (23%), *V. dahliae* (25%), *F. solani* (21%), *Monosporascus cannonballus* (15%), and *Rhizoctonia solani* (7%). *F. oxysporum* and *F. equiseti* were infrequently isolated. *M. cannonballus* was found frequently in the southern San Joaquin Valley and in the Imperial Valley, less frequently in the upper San Joaquin Valley, and never in the Sacramento Valley. In contrast, *A. cucurbitacearum* was isolated only once in the Imperial Valley and frequently in the central and northern production areas of the San Joaquin and Sacramento Valleys. The incidence of *V. dahliae* was concentrated in the production areas of Fresno and Merced Counties, occurring only rarely outside these areas. The other fungi exhibited a more general distribution.

Distinct symptoms often were associated with specific fungi. For example, *A. cu-*

curbitacearum was associated with yellowish or brownish corky lesions, which often formed discrete transverse bands on the tap and secondary roots (Table 1, Fig. 1A and B). *Rhizopycnis vagum* also was isolated occasionally from these lesions. Pycnidia of *R. vagum* were found occasionally on symptomatic roots that were incubated in a moist chamber. *R. vagum* also was isolated from pinkish, sunken lesions on the crown of the vine (Fig. 1C), and from generally discolored or rotten roots (Table 1). *M. cannonballus* was associated with reddish-brown lesions of the tap and secondary roots and necrosis of secondary and tertiary roots. Perithecia of *M. cannonballus* often were observed on such tertiary roots. *Macrophomina phaseolina* was associated with a wet, darkly discolored rot of the secondary and tap roots and with crown lesions, where microsclerotia, characteristic of this fungus, were embedded in the diseased tissue. *V. dahliae* was isolated from stem vascular tissue that was lightly discolored. Tissues exhibiting a brownish, wet decay or dark discoloration were plated on the selective medium and often yielded *Pythium* spp. *F. solani* was associated with the discrete reddish lesions, as well as with corky lesions, but these symptoms were not reproduced in subsequent pathogenicity tests (*data not presented*). *F. equiseti* was isolated from roots with various symptoms, as well as from asymptomatic tissues, but all *F. equiseti* isolates were non-pathogenic to cantaloupe (*data not presented*).

An average of 1.4 ascospores/g of soil characteristic of *M. cannonballus* were

recovered from 21 soil samples taken from six counties. Spores were recovered from all samples, with a range from 0.15 to 5.85 ascospores/g of soil. Neither their viability nor their pathogenicity was confirmed.

Field pathogenicity tests. In all 3 years, *M. cannonballus* caused a significant level of root rot, while the symptoms caused by the other fungi were moderate to none. In 1997, *M. cannonballus* caused a severe root rot ($P = 0.0001$) and reduced root length density by 93% ($P = 0.048$; Table 2). In 1995 and 1996, the levels of disease caused by *M. cannonballus* were similar (*data not presented*). In 1995, *V. dahliae* caused plant collapse in all inoculated plots but no root symptoms. In 1996, the metam-sodium treatment prior to soil inoculation had no effect on subsequent disease severity (mean disease severity of 0.8 in treated plots and 0.7 in non-treated plots). Nor did differences in the irrigation regime produce different results (mean disease severity of 0.7 in the first experiment and 1.0 in the second). In all 3 years, *M. cannonballus* caused discrete red lesions, a general rot of secondary and lower tap roots, and vascular discoloration of plants grown in infested soil. Perithecia of *M. cannonballus* were visible on many dead secondary and tertiary roots. In all 3 years, *A. cucurbitacearum* caused corky lesions on roots (Fig. 1D). *F. solani* did not cause any disease symptoms. *R. vagum* caused a slight pinkish-brown root and hypocotyl discoloration. The inoculated fungi were reisolated from roots in all treatments of these experiments.

Greenhouse pathogenicity tests. In a preliminary experiment, three isolates of *R.*

Table 2. Effect of fungi on cantaloupe root health, 1997 field microplot trial^w

Fungus ^x	Root disease index ^y	Root density (cm/cm ³)
<i>Monosporascus cannonballus</i>	2.9 a	0.07 b
<i>Acremonium cucurbitacearum</i>	0.8 b	0.45 ab
<i>Rhizopycnis vagum</i>	0.4 b	1.05 a
None	... ^z	1.00 a

^w Means in each column followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's least significant difference test. Values are means of three observations in one trial; results of two other trials were similar.

^x Inoculum levels in CFU/g of soil: 400 for *M. cannonballus*, 3,000 for *A. cucurbitacearum*, and 6,500 for *R. vagum*.

^y 0 = no symptoms; 1 = few lesions (covering <10% of root), secondary root rot slight; 2 = rot of secondary roots or lesions covering approximately 25% of the root; 3 = lesions covering at least 50% of the root, dead secondary roots; and 4 = general root rot, most of the root affected.

^z No disease observed on roots grown in non-infested soil; not included in analysis.

Table 1. Frequency of isolation of various fungi from melon roots collected from 81 fields over a 3-year period^z

Root tissue and symptoms	<i>Rhizop. vagum</i>	<i>A. cucurb.</i>	<i>Monosp. cannon.</i>	<i>V. dahliae</i>	<i>Pythium spp.</i>	<i>Macro. phaseo.</i>	<i>R. solani</i>	<i>F. solani</i>	Total
Light brown tap or secondary root	3	21	0	0	1	3	2	4	34
Brown to dark brown tap or secondary root	5	14	1	0	6	8	3	1	38
Soft, watery rot of any part of root system	6	5	6	0	12	10	1	2	42
Discrete, red lesions on tap and secondary root	5	5	11	0	1	2	0	8	32
Corky lesions/Banding on tap and secondary root	28	134	0	0	4	4	8	10	188
Discolored vasculature	4	1	10	22	0	0	0	3	40
Asymptomatic root tissues	7	9	1	1	2	4	0	3	27

^z Fungal species, from left to right: *Rhizopycnis vagum*, *Acremonium cucurbitacearum*, *Monosporascus cannonballus*, *Verticillium dahliae*, *Pythium* spp., *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium solani*, total number of isolations.

vagum reduced cantaloupe dry weight by 90% ($P = 0.0001$) and caused root discoloration ($P = 0.007$; data not presented). In a subsequent experiment, a California isolate of *R. vagum* reduced dry weight of the cucurbits by an average of 19.1% ($P = 0.0025$), with significant reductions to cantaloupe (cv. Top Net) and watermelon (Fig. 2). In a replicate trial in which the plants grew less vigorously, presumably due to lower average greenhouse temperatures, dry weight of each of the five cultivars was reduced ($P = 0.0001$, data not presented). In all experiments, *R. vagum* caused root discoloration and corky lesions on hypocotyl tissue (Fig. 1F), which were more severe on the melons and watermelons than on squash ($P = 0.001$, Table 3).

In two trials, *A. cucurbitacearum* reduced dry weight and shoot length of the cucurbits by an average of 11% ($P = 0.0065$) and 12% ($P = 0.0098$), respectively. Dry weight of cantaloupe (cv. Magnum .45) and shoot length of honeydew were significantly reduced (Fig. 3). On the melons and watermelon, *A. cucurbitacearum* caused a general discoloration of

the root system and a corky rot of the hypocotyl (Fig. 1E, Table 3). No disease was observed on squash or on any control plants.

In one trial, *M. cannonballus* reduced dry weight and shoot length of the cucurbits by an average of 42.3% ($P = 0.0001$) and 42.9% ($P = 0.0001$), respectively. Only growth of honeydew was not reduced significantly (Fig. 4). Disease symptoms included a general rot of the lateral roots and lesions on the tap root cortex. Symptoms were more severe on the melons and watermelon than on squash ($P = 0.0002$, Table 3). In a replicate trial, *M. cannonballus* significantly reduced the shoot length and dry weight of the inoculated plants by an average of 10% ($P = 0.031$) and 12% ($P = 0.0087$), respectively (data not presented).

DISCUSSION

The number of pathogenic fungi capable of colonizing melon roots and the similarity of the symptoms they induce confounded diagnosis based on the appearance of roots. A few very distinctive signs, such as the microsclerotia of *Macrophomina phaseolina* embedded throughout diseased tissue, and the erumpent perithecia of *Monosporascus cannonballus* on dead secondary and tertiary roots, aided in the diagnosis of some root rots. In the absence of such signs, diagnosis necessitated observation of fungal sporulation on surface-sterilized tissue or isolation of the fungus in culture.

Several fungi were isolated from symptomatic roots of melons in California production areas, including *Macrophomina phaseolina*, *A. cucurbitacearum*, *Monosporascus cannonballus*, *Rhizopycnis vagum*, *V. dahliae*, *F. solani*, and *Pythium* spp. Although the frequency of isolation of a given organism varied with the symptomatology, there was a substantial overlap of symptoms associated with different fungi. Frequently, more than one fungus was isolated from a single lesion or root. For example, *A. cucurbitacearum* and *R. vagum* were often isolated concomitantly. In their survey of four melon fields suffering vine decline in Texas, Merteley et al. isolated *M. cannonballus*, *F. solani*, *F.*

oxysporum, a *Stagonospora* sp., *Macrophomina phaseolina*, *Pythium* spp., and a *Cephalosporium* sp., but only *Monosporascus cannonballus* and *Macrophomina phaseolina* were pathogenic to cantaloupe in greenhouse experiments (15). In Spain, *A. cucurbitacearum* was concluded to be the causal agent of sudden death of melons, but *M. phaseolina*, *Rhizoctonia solani*, *Monosporascus eutypoides*, *Phytophthora* spp. and *Pythium* spp. were also isolated (10). Of these, only *A. cucurbitacearum* and *R. solani* were reported as being pathogenic to melon under their test conditions (10).

Isolates of *F. solani* from cantaloupe and honeydew were nonpathogenic to melons in our greenhouse experiments and were apparently secondary colonizers of diseased tissue. Only a single isolate from rotted watermelon roots was pathogenic. Champaco et al. (8) also isolated *F. solani* from rotted root of melons in Texas, but could not demonstrate pathogenicity under greenhouse conditions (8,15). *F. equiseti* likewise was not pathogenic in our tests, though it is known to cause disease under certain conditions (1). Both *F. solani* and *F. equiseti* are common soil saprophytes and are frequently isolated from roots (11).

V. dahliae was isolated from vascular tissue of declining vines and caused a collapse of inoculated plants in field microplots, but did not cause any root symptoms. *V. dahliae* was isolated most frequently in Merced County, where rotation of melons with cotton may contribute to its prevalence. In some fields, vascular colonization by *V. dahliae* may contribute to vine decline.

In our work, *M. cannonballus* caused severe root rot of melons in field microplots. However, *M. cannonballus* was isolated from diseased plants in only a few fields in our survey. Symptoms or signs of *Monosporascus* root rot were observed commonly in growing areas in the Imperial Valley and lower San Joaquin Valley, rarely in the upper San Joaquin Valley, and never in the Sacramento Valley. Although ascospores characteristic of *M. cannonballus* were retrieved from soils in the Sacramento Valley, it was never isolated

Fig. 2. Effect of *Rhizopycnis vagum* on shoot length and dry weight of greenhouse-grown cucurbits. Abbreviations: wa = watermelon cv. Crimson Sweet, hd = honeydew cv. Green Flesh, m45 = cantaloupe cv. Magnum .45, tn = cantaloupe cv. Top Net, and sq = squash cv. Super Set. Bars indicate the standard error of the mean of four observations in one trial. Asterisks indicate differences between adjacent treatments significant at $P \leq 0.05$.

Table 3. Effect of various fungi on root health of greenhouse-grown cucurbits

Cucurbit cultivars	Root disease index ^y		
	<i>Rhizopycnis vagum</i>	<i>Acremonium cucurbitacearum</i>	<i>Monosporascus cannonballus</i>
Watermelon cv. Crimson Sweet	2.6 a	1.4 a	2.4 a
Honeydew cv. Green Flesh	2.6 a	1.8 a	1.8 a
Cantaloupe cv. Magnum .45	2.6 a	1.4 a	2.4 a
Cantaloupe cv. Top Net	2.6 a	1.6 a	2.2 a
Squash cv. Super Set	1.5 b	0.0 ^z	0.5 b

^y 0 = no symptoms; 1 = slight or limited area of discoloration; 2 = general discoloration, one or two lesions; 3 = general discoloration, rot of some tissues; and 4 = rot of entire tap and feeder roots. Means in each column followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's least significant difference test. Values are means of eight observations in two trials.

^z No disease observed, not included in analysis of variance.

from plants, nor were typical symptoms ever seen in that area. It is possible that *M. cannonballus* is persisting saprophytically in this area or that the recovered spores were not those of *M. cannonballus*. Due to its thermophilic nature (14,23), *M. cannonballus* may be more active as a pathogen in southern California.

A. cucurbitacearum and *Rhizopycnis vagum* caused only slight root rot on melons in field microplots, but caused a more severe secondary root rot under greenhouse conditions. In contrast, symptoms caused by *M. cannonballus* were more severe in the field than in the greenhouse. This discrepancy in disease severity between the two types of experiments may be due to differences in inoculum potential, or interactions with environmental conditions (soil type, irrigation, temperature, and so on).

Although *A. cucurbitacearum* has been reported from California (2,5), this is the first documentation of its frequent and widespread occurrence throughout the state. The observed resistance of summer squash (*C. pepo*) to *A. cucurbitacearum* adds to the list of known resistant cucurbits, which includes autumn squash (*C.*

maxima, 4). Our results from the greenhouse studies confirm previous reports of the susceptibility of cantaloupe to *R. vagum* (9,16). This is the first report of *R. vagum* in California.

Although root colonization by *A. cucurbitacearum*, *R. vagum*, and *M. cannonballus* may contribute to vine decline, there are likely to be other factors that determine whether a vine collapses or only sustains root damage and still produces a profitable yield. In our survey, plant pathogenic fungi were isolated from vines exhibiting root symptoms but with healthy foliage, suggesting that some root damage can be tolerated by the vine, at least under certain conditions. In Israel, it has been shown that heat stress and fruit load stress may be significant factors affecting vine decline of melons, in addition to root infection by fungi (19). The environmental factors that contribute to the disease in California need to be determined.

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LITERATURE CITED

- Adams, G. C., Jr., Gubler, W. D., and Grogan, R. G. 1987. Seedling disease of muskmelon and mixed melons in California caused by *Fusarium equiseti*. Plant Dis. 71:370-374.
- Alfaro-García, A., Armengol, J., Bruton, B. D., Gams, W., García-Jiménez, J., and Martínez-Ferrer, G. 1996. The taxonomic position of the causal agent of Acremonium collapse of muskmelon. Mycologia 88:804-808.
- Amann, A. B. 1989. The etiology of Pythium sudden wilt of cantaloupe in the Imperial Valley of California. MS thesis. University of California, Davis.
- Armengol, J., Sanz, E., Martínez-Ferrer, G., Sales, R., Bruton, B. D., and García-Jiménez, J. 1998. Host range of *Acremonium cucurbitacearum*, cause of Acremonium collapse of muskmelon. Plant Pathol. 47:29-35.
- Bruton, B. D., Davis, R. M., and Gordon, T. R. 1995. Occurrence of *Acremonium* sp. and *Monosporascus cannonballus* in the major cantaloupe and watermelon growing areas of California. (Note) Plant Dis. 79:754.
- Bruton, B. D., Gordon, T. R., and Davis, R. M. 1995. Optimum CFU concentrations for testing pathogenicity of California cucurbit isolates of *Monosporascus cannonballus* and an *Acremonium* sp. (Abstr.) Phytopathology 85:1119.
- California Agricultural Statistics Service. 1999. Calif. Veg. Rev. 20.
- Champaco, E. R., Martyn, R. D., Barnes, L. W., Miller, M. E., Amador, J. M., and Perez, A. 1988. Root rot, a new disease of muskmelon in South Texas. (Abstr.) Phytopathology 78:626.
- Farr, D. F., Miller, M. E., and Bruton, B. D. 1998. *Rhizopycnis vagum* gen. et sp. nov., a new coelomycetous fungus from roots of melons and sugarcane. Mycologia 90:290-296.
- García-Jiménez, J., Velázquez, M. T., Jordá, C., and Alfaro-García, A. 1994. *Acremonium* species as the causal agent of muskmelon collapse in Spain. Plant Dis. 78:416-419.
- Gordon, T. R., Okamoto, D., and Jacobson, D. J. 1989. Colonization of muskmelon and non-susceptible crops by *Fusarium oxysporum* f. sp. *melonis* and other species of *Fusarium*. Phytopathology 79:1095-1100.
- Gottlieb, M., and Butler, K. D. 1939. A Pythium root rot of cucurbits. Phytopathology 29:624-628.
- Gubler, W. D., and Grogan, R. G. 1982. Factors affecting sudden wilt of melon in California. (Abstr.) Phytopathology 72:986.
- Martyn, R. D., and Miller, M. E. 1996. *Monosporascus* root rot and vine decline: an emerging disease of melons worldwide. Plant Dis. 80:716-725.
- Mertely, J. C., Martyn, R. D., Miller, M. E., and Bruton, B. D. 1991. Role of *Monosporascus cannonballus* and other fungi in a root rot/vine decline of muskmelon. Plant Dis. 75:1133-1137.
- Miller, M. E., Bruton, B. D., and Farr, D. F. 1996. Association of a Stagonospora-like fungus on roots of melons exhibiting vine decline symptoms. (Abstr.) Phytopathology 86:S3.
- Nelson, P. E., Tousson, T. A., and Marasas, W. F. O. 1983. *Fusarium* Species: An Illustrated Manual for Identification. The Pennsylvania State University Press, University Park.
- Newman, E. I. 1966. A method of estimating the total length of root in a sample. J. Appl. Ecol. 3:139-145.
- Pivonia, S., Cohen, R., Kafkafi, U., Ben Ze'ev, I. S., and Katan, J. 1997. Sudden wilt of melons in southern Israel: Fungal agents and relationship with plant development.

Fig. 3. Effect of *Acremonium cucurbitacearum* on shoot length and dry weight of greenhouse-grown cucurbits. Abbreviations: wa = watermelon cv. Crimson Sweet, hd = honeydew cv. Green Flesh, m45 = cantaloupe cv. Magnum .45, tn = cantaloupe cv. Top Net, and sq = squash cv. Super Set. Bars indicate the standard error of the mean of eight observations in two trials. Asterisks indicate differences between adjacent treatments significant at $P \leq 0.05$.

Fig. 4. Effect of *Monosporascus cannonballus* on shoot length and dry weight of greenhouse-grown cucurbits. Abbreviations: wa = watermelon cv. Crimson Sweet, hd = honeydew cv. Green Flesh, m45 = cantaloupe cv. Magnum .45, tn = cantaloupe cv. Top Net, and sq = squash cv. Super Set. Bars indicate the standard error of the mean of four observations in one trial. Asterisks indicate differences between adjacent treatments significant at $P \leq 0.05$.

- Plant Dis. 81:1264-1268.
20. Pollack, F. G., and Uecker, F. A. 1974. *Monosporascus cannonballus*, an unusual Ascomycete in cantaloupe roots. Mycologia 66:346-349.
 21. Puhalla, J. E. 1984. A visual indicator of heterokaryosis in *Fusarium oxysporum* from celery. Can. J. Bot. 62:540-545.
 22. Risser, G., and Laugie, M. 1968. Mise en evidence de la sensibilite de divers cultivars de melon (*Cucumis melo* L.) a *Pyrenochaeta* sp agent de la "maladie des racines liegeuses" de la tomate. Ann. Amelior. Plantes 18:75-80.
 23. Stanghellini, M. E., Kim, D. H., and Rasmussen, S. L. 1996. Ascospores of *Monosporascus cannonballus*: Germination and distribution in cultivated and desert soils in Arizona. Phytopathology 86:509-514.
 24. Stanghellini, M. E., and Rasmussen, S. L. 1992. A quantitative method for the recovery of ascospores of *Monosporascus cannonballus* from field soil. (Abstr.) Phytopathology 82:1115.
 25. Troutman, J. L., and Matejka, J. C. 1970. Three fungi associated with cantaloupe roots in Arizona. (Abstr.) Phytopathology 60:1317.
 26. Ward, K. J., Klepper, B., Rickman, R. W., and Allmaras, R. R. 1978. Quantitative estimation of living wheat-root lengths in soil cores. Agron. J. 70:675-677.