Acremonium Species as the Causal Agent of Muskmelon Collapse in Spain

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ABSTRACT

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Over the last decade, a new disease of muskmelon (*Cucumis melo*) has appeared in the coastal Mediterranean areas of Spain where early melons are produced in the field. The economic losses are severe, and the disease is spreading to new areas. The aim of this research was to describe the disease and conduct Koch's postulates to show an *Acremonium* sp. to be the causal agent. This fungus has not previously been reported to cause a similar disease on melon. The obvious aboveground symptom of the disease is a sudden collapse of the plant when the first fruit is ripening. Root symptoms appear soon after planting and include yellow discoloration and corking of the upper root, generalized death of secondary roots, and continuous production of superficial roots. Characteristically, there are no aerial symptoms, plant decline, stunting, or slow growth until wilting occurs. The disease may be named Acremonium melon collapse to avoid confusion with any other disease characterized by a sudden nonvascular wilt.

Muskmelon (*Cucumis melo* L.) is one of the more important horticultural crops in Spain, with 900,000 t produced on 70,000 ha. Over the last 10 yr, a sudden death syndrome has become prominent in areas of the Spanish Mediterranean eastern coast where most of the early-melon field production occurs. The economic importance of this problem is considerable; according to a 1989 estimate in the Valencia area, infested fields had approximately 30% plant mortality at harvest (8). An evaluation of the disease impact on the affected melon-producing areas has indicated that 25% of infested fields were removed from muskmelon production during the last 4 yr (A. Miguel, *personal communication*).

The most obvious symptom of the disease is a nonvascular wilt or collapse of the plant as the first fruits approach maturity. At this time, the principal roots are corked and exhibit many light-brown lesions at the juncture of secondary and tertiary roots, which have generally disappeared. Most of the remaining young roots are produced from the base of the stem. Plants with good development and growth die suddenly within a few days. No vine decline, stunting, slow growth, or damping-off takes place. The objectives of this study were to: 1) describe the foliar and root symptomatology of the disease, 2) determine the frequency and identity of the most prominent fungi isolated from affected muskmelon roots representing the major production areas, and 3) study the pathogenicity of the most prominent fungi and, using Koch's postulates, determine the causal agent of melon collapse. A previous report has been published (9).

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MATERIALS AND METHODS

Symptom development. Eleven different fields around the Valencia area were visited periodically for 2 yr to examine root and foliar symptoms along the muskmelon crop cycle. The fields included some supplementary melon plants so that plants could be removed periodically and development of root symptoms observed. Periodic pathogenicity tests compared plants grown in naturally infested or in sterilized soil inoculated or not inoculated with candidate fungal strains.

Sampling and fungal isolation. A total of 32 fields in which well-characterized symptoms of melon collapse developed were selected in eastern (Valencia, Cataluña, and Baleares), southeastern (Murcia), and central (Castilla-La Mancha) regions of Spain. Melon plants from each field were systematically analyzed for root fungal population. Plants from six fields that had never had collapse were used as controls. As an additional control, melon plants were grown in steam-sterilized soil (1.5 atm, 1 hr) from representative infested fields. Isolations were made from plant roots from affected fields. When the root systems were too deteriorated, fungi were isolated from seedlings of the susceptible cultivar Piel de Sapo grown in suspect field soil for 40 days in the greenhouse. More than 10 plants ($\bar{x}$ = 25.4 plants) were individually studied for fungal populations from each collapse-affected field as well as from fields that had no history of collapse.

For isolation, roots were washed under running tap water for 1 hr, surface-sterilized for 1 min in a 1.5% sodium hypochlorite solution, and washed twice with sterile water. Several root fragments were plated on agar media. A systematic study was made with various solid media: oat agar, potato-dextrose agar, malt agar, water agar, Komada's medium, cornmeal agar 3P (6), and potato-dextrose agar supplemented with 500 μg/ml of streptomycin sulfate (PDAS). PDAS was finally adopted as the standard isolation medium. Cultures were incubated in the dark at 25–27 C for 4–6 days, and colonies were identified when possible. Unidentified fungi were transferred to fresh PDA plates and incubated at room temperature under NUV + fluorescent illumination, 12-hr photoperiod. The isolates were maintained until they sporulated, then were classified and transferred to a tube culture collection for further study. Most of the isolates sporulated in a few weeks, but some strains of *Monosporascus* took longer. After 2.5 mo of observation, the fungal strains that failed to sporulate were labeled as nonsporulating fungi. The identification of *Acremonium* sp. was confirmed by W. Gams at the Dutch Central Bureau of Schimmelcultures and M. A. J. Williams at the International Mycological Institute.

Pathogenicity tests. Pathogenicity tests of the most prominent fungal isolates were conducted in 25-L pots filled with autoclaved sandy soil. Each pot was inoculated with a 20-day-old PDA culture of the fungal isolate to be tested and thoroughly mixed with the soil. Each pot was sown with six surface-sterilized seeds of cv. Piel de Sapo and kept in a greenhouse at 16–28 C for 80 days. Each treatment was replicated twice, and controls with uninoculated plants in sterilized soil and in naturally infested soils were included. One plant of each pot was systematically removed at about 10-day intervals to evaluate symptom development. With this procedure, sequential root symptom development, periodic reisolation, and final overall symptomatology could be evaluated in order to fulfill Koch's postulates. To ascertain pathogenicity of melon *Acremonium* sp. isolates, the host list of the Shasta daisy (*Chrysanthemum maximum* Ramond) wilt pathogen, *A. strictum* W. Gams (5), was evaluated. Four isolates of *Acremonium* sp. shown to be pathogenic on melon were each inoculated onto Shasta daisy, okra (*Hibiscus esculentus* L.), and cotton (*Gossypium hirsutum* L.), as above. Melon was also included as a control. Two replicates were used for each strain-host combination. In addition, two other replicates were planted

in naturally infested soil and sterilized soil. Plants were grown in a greenhouse for 3 mo.

A simple hydroponic test to evaluate short-term pathogenicity responses was developed. Surface-disinfected seeds of cv. Piel de Sapo were germinated and grown in steam-sterilized vermiculite until the cotyledons were fully expanded. At this stage, batches of five to seven plants were transplanted to 1-L containers where roots were submerged in an aerated, sterilized half-strength Hoagland solution supplemented with one triturated PDA culture grown as before. Containers were maintained at room temperature (18–27 C) under natural light, and plants were scored after 8 days. A positive test resulted if the plant tops wilted and roots were rotten.

RESULTS

Symptom development. Root symptoms started very early in the affected fields. In direct seeding, the seedling hypocotyl showed light yellow-brown discolorations (Fig. 1) that later developed into dry, corky brown areas, and the rootlets became discolored and then necrotic. This rootlet destruction was an extensive process and could be clearly discerned in the transplanted plants. When young transplanted plants were produced on collapse-free soil, the root symptoms could not be seen at 2 wk after transplanting and the roots retained soil when plants were dug up; nevertheless, careful examination showed discolored lesions on these rootlets. Two weeks later, these roots appeared soil-free due to necrosis of the secondary roots and radicular hairs (Fig. 2). The plant produced new rootlets above the affected ones (Fig. 1). This process occurred continuously, and finally main roots appeared bare and commonly had lesions at the points where the secondary roots had disappeared (Fig. 3). Later, small outgrowths occurred in the area of the lesions so that the root of a mature plant appeared to have transverse rings. Corky areas in the crown area of the plant sometimes developed localized rot.

The affected plants usually did not show any abnormal foliar symptoms until fruit ripening began. At this time, interveinal yellowing of older leaves and then lack of turgidity in young leaves were seen within 4–6 days. Affected plants showed the definitive symptoms that have given rise to the names for this disease in Spain: *colapso* (collapse) and *muerte súbita* (sudden death). Vines became flaccid, leaves turned brown, and the whole plant wilted and died. No vascular symptoms developed. Fruits appeared healthy but were tasteless and frequently smaller than normal, failing to reach a commercial size. Occasionally, they showed external sunburn areas.

Subsequent melon crops are severely affected if planted in fields with a history of the disease. Disease distribution in the field does not have an established pattern. The problem starts in isolated plants in some fields and as patches of affected plants in others, with some quantitative differences according to the year. However, the final result is extensive plant death.

Over the 5 yr of this study, the disease appeared to be host-specific and the affected fields could successfully produce other horticultural crops. Even watermelons were cultivated with good results in many places as an alternate crop on seriously infested soils. Nevertheless, this fact must be accepted cautiously, because in some soils, watermelon is being affected by a similar disease with slower development of symptoms. Cucumber is affected very slightly or not at all by the disease.

Causal agent isolation. Root isolation frequencies for the most important fungal species obtained from 32 fields with symptoms of collapse and from six with no history of collapse are shown in Table 1. The characteristic *Acremonium* sp. was always isolated from plants in fields affected by collapse. Conversely, the species was never isolated from melon plants from disease-free fields or from plants grown in containers with infested sterilized soils. This fungal agent was isolated only from the roots. Numerous *Fusarium* spp. were isolated, including *F. oxysporum* Schlechtend.:Fr. and *F. solani* (Mart.) Sacc., but neither was capable of reproducing the disease. Other potential pathogens of melon were present at different frequencies: *Macrophomina phaseolina* (Tassi) Goidanich, *Rhizoctonia solani* Kühn, and *Monosporascus eutypoides* (Petrak) von Arx were isolated from 37, 28, and 19% of the affected soils, respectively. *Phytophthora* spp. were present in only 6% of the affected soils. Several *Pythium* spp. were isolated in 56% of the affected soils, but no consistent isolation pattern could be ascertained among the different species obtained, the most common being *P. aphanidermatum* (Edson) Fitzp., which was isolated in only a few cases. Different regions with different cropping histories had differences in the frequency of these fungi, but none of these fungi was isolated in 21.9% (seven of 32) of the affected soils; this increased to 37.5% when the heterogenous *Pythium* group was not considered.

Other fungi, including species of *Chaetomium, Penicillium*, and *Alternaria*,

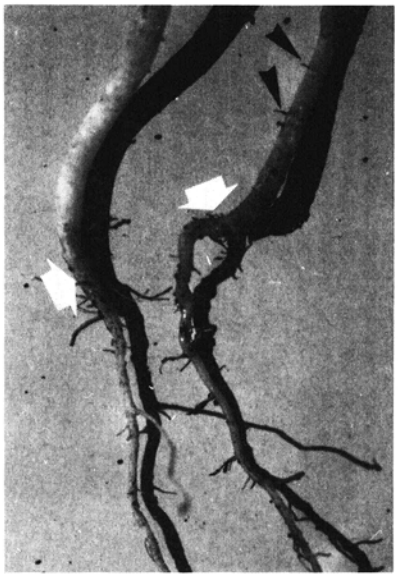

Fig. 1. First symptoms of collapse in muskmelon seedlings include light yellow-brown discolorations at the hypocotyl (white arrows) and production of new roots above the affected zone (black arrows).

Fig. 2. Muskmelon plants produced in a sterilized nursery and transplanted to infested soil: (Right) A 2-wk-old plant with hairy roots that retain the soil and (left) a 4-wk-old plant with soil-free roots and no fine rootlets.

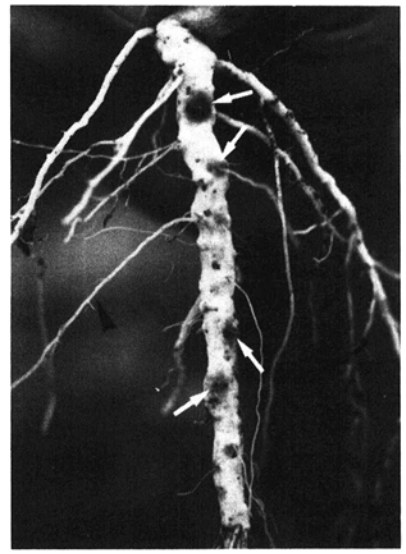

Fig. 3. Muskmelon root grown for 2 mo in infested soil shows necrosis at insertion points of disappeared secondary roots (white arrows) and sparse ramification of others (black arrow).

Table 1. Identities and frequencies of the most representative fungi[a] isolated from muskmelon roots collected from fields in several regions of Spain

	Isolation frequency (%)[b]						
	Farms with muskmelon collapse[c]						Farms without muskmelon collapse[d]
Fungus	Valencia	Murcia	Castilla-La Mancha	Cataluña	Baleares	Av.	
Acremonium sp.	100	100	100	100	100	100	0
Fusarium spp.	100	100	100	100	100	100	83
Pythium spp.	50	33	80	100	50	56	50
Macrophomina phaseolina	19	50	60	33	100	37	33
Monosporascus eutypoides	31	17	0	0	0	19	0
Rhizoctonia solani	44	17	20	0	0	28	0
Phytophthora spp.	12	0	0	0	0	6	0

[a]Fungi isolated at low frequencies included *Alternaria, Helminthosporium* complex, *Penicillium, Aspergillus, Botrytis,* and *Chaetomium* spp.
[b]Percentage of farms with positive isolations of the fungus.
[c]Sixteen in Valencia, six in Murcia, five in Castilla-La Mancha, three in Cataluña, and two in Baleares.
[d]Six in Murcia.

Table 2. Pathogenicity of the fungi most commonly isolated from the roots of plants with melon collapse

			Muskmelon[a] pathogenicity	
Fungus	Isolates	Origin	Infested soil[b]	Hydroponic test[c]
Acremonium sp.	A-49[d]	Valencia	+[e]	+[f]
	A-99[d]	Valencia	+	+
	A-69	Murcia	+	+
	A-243	Castilla-La Mancha	+	+
	A-410	Murcia	+	+
	A-419	Castilla-La Mancha	+	+
	A-462	Cataluña	+	+
	A-Sol	Valencia	+	+
	A-Alc	Valencia	+	+
Monosporascus eutypoides	C-1	Murcia	−	−
	C-2	Valencia	−	−
	C-18	Valencia	−	−
	C-28	Murcia	−	−
	C-29	Valencia	−	−
Macrophomina phaseolina	M-67	Valencia	−	−
	M-B	Castilla-La Mancha	−	−
Rhizoctonia solani	R-Alc	Valencia	+[g]	+[f]
	R-48	Murcia	+	+

[a]Susceptible cultivar Piel de Sapo.
[b]Pots filled with autoclaved sandy soil were inoculated with one 10-cm petri dish of 20-day-old fungal culture on PDA and kept in a greenhouse at 16–28 C.
[c]Seedlings with fully expanded cotyledons were put in 1-L jugs with aerated, sterilized half-strength Hoagland solution and inoculated. Plants were scored after 8 days at room temperature (18–27 C).
[d]Monosporic.
[e]All *Acremonium* sp. isolates reproduced collapse symptoms in infested soil.
[f]All *Acremonium* sp. and *R. solani* isolates produced root rot and wilt.
[g]Symptoms differed from those of muskmelon collapse.

Fig. 4. Conidiophores and conidia of *Acremonium* sp. from PDA culture.

were isolated occasionally. Nonsporulating fungi were also isolated but were generally inconsistent and infrequent.

Pathogenicity tests. Of the fungi tested, only *Acremonium* sp. and *R. solani* proved to be pathogenic on melon (Table 2). Only *Acremonium* sp. reproduced collapse symptoms and also the sequential symptoms from the seedling stage to collapse at plant maturity (75–85 days). After approximately 10 days, the transition region between the hypocotyl and root exhibited distinct yellow-brown lesions. By 20 days, the transition region had developed a corky appearance and small rootlets were often necrotic; during this same period, adventitious roots typically started to be produced on the hypocotyl. The destruction of the rootlets progressed farther up, giving a bare root appearance, with no foliar symptoms until the end of the period. No symptoms were observed on cotton, okra, or Shasta daisy grown in naturally infested soil or in soil inoculated with *Acremonium* sp.

Inoculation with *R. solani* produced seriously diseased plants that developed a dry rot of the crown and died in a short time but did not show the characteristic symptoms. This disease and the crown soft rot of melon produced by *Pythium aphanidermatum* are known and have been documented recently in Spain (19). Because *F. oxysporum* and *F. solani* were commonly isolated, isolates of both species were extensively tested for pathogenicity in the preliminary phase of this work. None of these isolates—110 and 69, respectively—were pathogenic to melon. Neither *Monosporascus eutypoides* nor *Macrophomina phaseolina* provoked any detectable disease symptoms under the conditions of these studies. Results of the hydroponic test with *Acremonium* sp. isolates were rapid and reproducible. Over 2 yr of experiments, this test produced comparable results with those in soil for the *Acremonium* sp. isolates checked by both tests (Table 2).

Description of the fungal agent. The *Acremonium* sp. causing melon collapse produced white, cottony colonies with reverse white cream to light honey color on PDA. Conidia were aggregated in slime heads, nonseptate, hyaline, oval to cylindrical with obtuse ends, measuring 3.8–11.0 × 1.5–4.0 μm ($\bar{x}$ = 6.2 × 2.8) (Fig. 4). Conidiogenous cells were monophialidic, subulate, measuring 20–97 × 1–2 μm ($\bar{x}$ = 50 × 1.5). Roughly rounded chlamydospores were very rare in agar cultures. Colonies reached a

diameter of 54 mm in 20 days at 27 C on PDA. No consistent differences in size or growth were observed among monosporic cultures. A teleomorph was never observed. The fungus was classified as *Acremonium* sp.

Isolates of this fungus are deposited at the Dutch Central Bureau of Schimmelcultures (CBS 683.88). *Monosporascus eutypoides* is deposited at the British International Mycological Institute (IMI 345135).

DISCUSSION

There are several melon diseases reported in the literature whose descriptions might be confused with that of Spanish melon collapse. A disease described in India caused a yellowing of the foliage, defoliation, and a vascular orange-red discoloration that extended on the stem for a small distance; it was caused by *F. solani* (21,18). A vine decline of melon has been attributed to *Macrophomina phaseolina* in Texas (4), Israel (16), and South Africa (15). In California, a sudden wilt of melon during the ripening stage was attributed to root attack by *Pythium* sp. (13). Another disease has been described in Israel (17) due to root infection by *Monosporascus eutypoides*. In the United States, Troutman and Matejka (20) described three fungal agents associated with roots of cantaloupe. Pollack and Uecker (14) described one of them as *Monosporascus cannonballus* Pollack & Uecker n. sp. (14), and in 1979 it was described from Japan (22). Recently, this fungus has been described associated with root rot/vine decline in Texas (12). A different and more complex case is a wilt studied in several cucurbits in Iran (1); the authors suggested that this syndrome resulted from the interaction of viral agents with radicular infections by *Fusarium, Phytophthora*, and *Macrophoma*.

The final symptoms of Spanish melon collapse on the foliage may be confused with those of other nonvascular wilts, many of which are similar in the advanced stages. However, this disease starts very early in the season and has an early root discoloration, progressive death of rootlets, and a sudden and complete process of wilting. None of the described cases mention this syndrome even in the latest revisions of cucurbit diseases (2,3). This symptomatology is evident to anyone who follows disease development in melon crops or in volunteer melon plants. The hypocotyl discoloration gives a differential and reproducible symptom for the disease caused by *Acremonium* sp. In practical terms, this point is critical because it establishes when the disease starts and gives a diagnostic procedure very early in the season (9).

No fungi other than *Acremonium* have been consistently associated with the disease, and *Acremonium* reproduced the disease in the different inoculation tests performed in this study. The inoculation trials in large containers reproduced the process found in the field, with the plant surviving the early root damage but collapsing when the fruit was expanding or ripening and maximum water was needed. In most affected areas in Spain, this crop stage is reached at the end of June or the first half of July, when the maximum annual temperature is reached.

The hydroponic test was developed after observing the high disease incidence in marsh soils that are flooded for several months each year and maintain a high water table during the crop. It was shown to be a quick and reliable test that gave a preliminary indication of plant resistance. For instance, *Benincasa hispida* (Thunb.) Cogn. and 14 hybrid rootstocks have been tested for resistance with this test, with *B. hispida* and eight of the hybrid rootstocks found to be resistant (7). All of the rootstocks found to be resistant were also resistant in naturally infested soils as intact plants or grafted with melon. This resistance is being used to produce melon in infested soils, since appreciable disease resistance does not appear to be present in commercial cultivars (*unpublished*).

Melon collapse seems to be limited to very few cucurbitaceous crops. In the affected fields, a study of common weeds showed that 41 botanical species belonging to 17 families were not affected by the disease and did not develop root lesions (10).

The Spanish melon *Acremonium* isolates share some characteristics with *A. strictum, A. sclerotigenum* (Valenta) W. Gams, and *A. crotocinigenum* (Schol-Schwarz) W. Gams (W. Gams, *personal communication*). Therefore, further taxonomic study is needed to establish the identity of this new pathogen. In 1982, a root and hypocotyl rot of young melons was described and attributed to *Cephalosporium cucurbitaerum* (11); the description does not fit with the Spanish *Acremonium* sp. from melon.

The length of time that this newly described disease has been present in melon crops in the Valencia area where the problem originated is unknown. It has spread to new areas, such as the Murcia Province, and appears to be the most destructive melon disease found in Spain.

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