Etiology, Incidence, and Distribution of Cotton Seedling Damping-off in Southern Spain

J. M. MELERO-VARA, Research Plant Pathologist, Departamento de Protección Vegetal, Dirección General de Investigación y Extensión Agrarias, Apdo. 240, 14080 Córdoba, and R. M. JIMÉNEZ-DÍAZ, Professor, Departamento de Agronomía, Escuela Técnica Superior de Ingenieros Agrónomos, Universidad de Córdoba, Apdo. 3048, 14080 Córdoba, Spain

ABSTRACT

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Disease surveys in 1980–1984 indicated widespread occurrence of cotton (*Gossypium hirsutum*) seedling damping-off in the Guadalquivir Valley of southern Spain. Dead seedlings occurred in 82–94% of the fields, with annual average incidences ranging from 6.6 to 9.4% dead seedlings per field. *Fusarium* spp., *Rhizoctonia solani* AG-4, *Pythium ultimum, Thielaviopsis basicola*, and *Phytophthora palmivora* MF1 A1, in order of decreasing frequency, were isolated from affected seedlings. Isolates of these fungi were pathogenic to cotton, except for those of *Fusarium*. *R. solani* AG-4 was the primary agent in the disease complex and was associated with severe necrosis of the root and/or hypocotyl. *Pythium ultimum* was associated with a watery decay of the root. *Phytophthora palmivora* MF1 A1 and *T. basicola* were minor components in the complex.

Additional keywords: *Fusarium equiseti, F. oxysporum, F. solani*

Upland cotton (*Gossypium hirsutum* L.) is a major component of the current cropping system for irrigated land of the Guadalquivir Valley in southern Spain. About 80,000 ha are sown annually to cotton in that region, which represents about 80% of the total national acreage of cotton. Because of lint losses frequently caused by rain in early fall, farmers often plant cotton early, which usually results in seedling damping-off and poor stands. Although the disease is widespread, little is known about its etiology and distribution in the Guadalquivir Valley.

Damping-off of cotton seedlings, a disease complex first described by Atkinson in 1892 in the United States (2) occurs worldwide. In the United States, estimated direct losses of annual yield of cotton due to damping-off ranged from 1.5 to 4% for the period 1953–1977 (16). Indirect losses also occur because of replanting costs, crop heterogeneity, and delay of harvest (12,17).

The etiology of damping-off of cotton seedlings is both complex and controversial. In many cases, most fungi isolated from infected tissues are weakly or not pathogenic, although some pathogenic fungi are recovered in very low frequencies (8,14). In general, *Rhizoctonia solani* Kühn, *Pythium ultimum* Trow, and *Thielaviopsis basicola* (Berk. & Br.) Ferr. are considered the most important pathogens in the complex (16,17). However, their relative importance may vary depending upon the environmental conditions and the soil type in the crop area. Most researchers agree that *Fusarium* spp. are of minor significance in the etiology of this disease, although they are frequently isolated from affected tissues (1,3,9,12,16). Nevertheless, *F. oxysporum* Schlecht., *F. equiseti* (Corda) Sacc., and *F. moniliforme* Sheld. are considered important components of the complex in some areas of the Cotton Belt of the United States (5,14,18).

This article presents research on the etiology, importance, and distribution of the seedling damping-off complex in the cotton-growing area of the Guadalquivir Valley in southern Spain. A preliminary report of a portion of this work has been published (11).

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MATERIALS AND METHODS

Disease surveys. Systematic disease surveys were conducted in the cotton-growing area of the Guadalquivir Valley during April and May of 1980 and 1982–1984. Seedlings were sampled at the cotyledon to six-true-leaf stage, with the two-true-leaf stage being the most prevalent. A total of 164 fields, mostly planted to the cultivar Coker 310, were inspected in a zigzag pattern, including 26, 44, 54, and 40 fields in 1980, 1982, 1983, and 1984, respectively. Incidence (percentage) of dead seedlings was assessed by counting the number of them in five groups of 50 consecutive seedlings chosen at random in each field. Except for 1982, occurrence of symptoms on underground tissues of otherwise symptomless plants was determined in a random sample of 50 plants uprooted from each field.

Isolation from affected seedlings. Seedlings affected with a variety of symptoms were sampled from each field and stored at 5 C for further observations and isolations in the laboratory. Isolations were made on 2% water agar amended with 30 μg/ml of aureomycin (WA), Difco potato-dextrose agar (PDA), and PDA acidified to pH 4.5–5.0 with lactic acid, on the day of sampling or shortly thereafter. Affected tissues were washed thoroughly under running tap water, cut into small pieces, surface-disinfested in 0.75% NaOCl for 1–2 min, blotted dry between sterile filter papers, and plated onto media. Plates were incubated in the dark at 18–24 C and observed daily for the development of fungal colonies. Young colonies were transferred to obtain pure cultures for identification and further studies. Isolates of *Fusarium* spp. were identified according to Booth (4).

Thirty-seven isolates of *Rhizoctonia* were paired against a tester of anastomosis group (AG) 4 (isolate 283, kindly provided by E. E. Butler, University of California, Davis). Pairings were made on thin films of WA on sterile microscope slides kept in moist chambers. Slides were incubated at 24–26 C in the dark until the mycelium overlapped (13). When isolates of *Rhizoctonia* spp. did not anastomose with the tester and mycelium was narrow (<4 μm wide), attempts were made to determine the nuclear condition using the staining technique described by Herr (7).

Isolates of *Phytophthora* spp. were identified at the Commonwealth Mycological Institute, Kew, Surrey, England.

Pathogenicity tests. The cotton cultivar Coker 310 was used in all pathogenicity tests unless otherwise stated. Seeds (25 or 36) were surface-disinfested, air-dried, germinated, and then sown in 25- × 25-cm flats filled with a soil mixture (sand and silt, 1:1, v/v) that had been fertilized with 40 g of Ca superphosphate (18%), 4 g of KNO_3, 4 g of K_2SO_4, 2 g of $CaCO_3$, and 1 g of $MgSO_4$ per 10 kg and infested with the appropriate

pathogen. Seedlings were grown in a growth chamber at 20 ± 2 C, with a 12-hr photoperiod of fluorescent light at 180 $\mu Em^{-1}sec^{-1}$ and were watered as needed. Pathogenicity was assessed by the percentage of pre- and postemergence damping-off and by the severity of symptoms in surviving plants. Incidence of postemergence damping-off was recorded at weekly intervals from 2 to 4–5 wk after planting unless otherwise stated. The surviving plants were uprooted and rated on a scale of 1–6 (1 = no symptoms, 2 = few necrotic lesions in root-hypocotyl, 3 = abundant necrotic lesions in roots or a few cankers in hypocotyl, 4 = extensive necrosis or several cankers in root-hypocotyl, 5 = severe and coalescent cankers in root-hypocotyl, 6 = dead seedlings). An average disease index was calculated for each flat. Analysis of variance and mean comparisons were performed on the data.

The pathogenicity of the isolates of *Rhizoctonia* spp. was tested by the method of Ko and Hora (10). Inoculum was prepared in an autoclaved (121 C for 1.5 hr, twice) mixture of chopped, peeled potato and sandy soil (1:10, w/w) in flasks (25 g per flask). The mixture in a flask was infested with small disks of mycelium from a culture on PDA and incubated at 26 ± 2 C in the dark for 2–3 wk. Flasks were shaken vigorously every 3–4 days to facilitate uniform colonization of the mixture. The colonized potato-soil mixture was thoroughly mixed with the nonsterile sand-silt mixture. Seeds were sown in flats filled with 6 kg of the infested soil. Control plants were grown in a mixture of uninfested potato-soil medium and nonsterile sand-silt soil.

The pathogenicity of four isolates of *R. solani* AG-4 and one binucleate *Rhizoctonia*-like isolate was first tested on the cotton cultivar Tabladilla 100 with rates of 17, 1.7, and 0.17 g of colonized potato-soil inoculum per kilogram of soil. One flat was used for each inoculum rate and isolate. Thereafter, the differences in the virulence of 30 isolates of *R. solani* AG-4 and seven isolates of binucleate *Rhizoctonia*-like isolates to Coker 310 were tested in a series of nine experiments with potato-soil inoculum at 1 g/kg of soil.

Inocula of three isolates of *Pythium ultimum* and two of *Phytophthora palmivora* (Butler) Butler were increased in an autoclaved (121 C for 1.5 hr, twice) cornmeal-sand mixture (1:9, w/w) (CMS) in flasks (300 g per flask) at 24 ± 1 C for 1 mo. Uniformly colonized CMS was thoroughly mixed with the sterile sand-silt mixture at 4, 10, and 40 g/kg of soil and planted. Control plants were grown in a mixture of uninfested CMS and sterile sand-silt soil. Two flats were used for each isolate and inoculum rate.

Initially, the pathogenicity of 152 isolates of *Fusarium* spp. was tested. Inoculation was accomplished either by placing a small disk of PDA from an actively growing colony of the fungus on the hypocotyl base of emerging cotton seedlings or by dipping the root of seedlings in a suspension of mycelium and conidia. Thirty-six selected isolates of *Fusarium* spp. that showed some pathogenicity in the initial test were evaluated again in infested soil. Inocula were increased in CMS at 25 ± 2 C for 3–5 wk and thoroughly mixed with sterile sand-silt soil at 50 g/kg of soil. Control plants were grown in a mixture of uninfested CMS and sterile sand-silt soil. One to three flats were used for each isolate. Plants were observed periodically for symptom development and indexed for disease 35–45 days after sowing.

The pathogenicity of isolates of *T. basicola* was tested by two inoculation methods. For one isolate, inoculum was increased in CMS at 25 ± 2 C for 45 days, then mixed with sterile sand-silt soil at 50 g/kg of soil. The pathogenicity of this isolate and another one was also tested as described by Tabachnik et al (15). Sterile sand-silt soil mixture was sprayed with a suspension of endoconidia at concentrations of 2×10^2, 2×10^3, and 2×10^4 per gram of soil and incubated in closed plastic bags at 20–25 C for 15 days. Seeds were sown in two flats of infested mixture for each treatment. Control plants were grown in mixtures of uninfested substrates. Plants were observed for symptom development and indexed for disease 45 days after sowing.

RESULTS

Disease symptoms in the field. A variety of symptoms were observed on cotton seedlings during disease surveys, including watery decay of roots, root and hypocotyl necroses, hypocotyl canker, and cotyledon collapse. Lack of seedling emergence was observed in some fields but no effort was made to determine its etiology.

Root and hypocotyl necroses were the most common symptoms. Necrosis either affected the entire root or was restricted to its distal part. Similarly, necrosis of the hypocotyl was restricted or extended to affect most of it. Frequently, lens-shaped, sunken, often girdling, necrosis (canker) occurred on the hypocotyl.

Table 1. Prevalence and incidence of cotton seedling damping-off in Andalucía, southern Spain

Year	Sampled fields[x] (no.)	Number of fields with incidence of dead seedlings[y]			Mean incidence of dead seedlings (%)
		Trace to 10%	11–20%	>20%	
1980	26	11	7	4	9.0
1982	44	30	4	3	6.6
1983	54	34	11	6	9.4
1984	40	25	5	3	7.3
Total	164	100	27	16	8.1

[x]Fields were randomly chosen along predetermined itineraries in the Guadalquivir Valley of Andalucía.
[y]Based on counts of dead seedlings in five groups of 50 consecutive seedlings per field.

Table 2. Symptomatology and frequency of isolation of fungi from cotton seedlings affected by damping-off in Andalucía, southern Spain[x]

Symptoms	Percentage of seedlings from which indicated fungi were isolated					
	Rhizoctonia solani	Pythium ultimum	Phytophthora palmivora	Fusarium spp.	Thielaviopsis basicola	Miscellaneous[y]
None	0.0	0.0	0.0	22.0	0.0	9.0
Restricted root necrosis	5.2	0.8	0.0	25.2	0.5	13.9
Extensive root necrosis	7.0	4.0	0.0	25.5	0.0	9.0
Watery decay of root	0.0	12.0	0.0	16.0	3.0	0.0
Extensive root and hypocotyl necrosis	12.3	0.3	0.7	34.1	1.3	9.3
Severe hypocotyl necrosis	10.3	1.0	1.3	25.7	0.0	7.7
Hypocotyl canker	4.7	0.3	0.0	24.0	0.0	9.7
Cotyledon collapse	0.0	0.0	0.0	4.5	0.0	21.0
Dead seedling	22.3	0.7	0.3	27.3	0.0	15.7

[x]Seedlings were sampled from 141 fields during disease surveys in 1980 and 1982–1984 in the Guadalquivir Valley of Andalucía.
[y]Miscellaneous category includes several genera of nonpathogenic fungi (species of *Alternaria*, *Chaetomium*, *Penicillium*, *Stachybotrys*, *Stemphylium*, etc.).

Desiccation of cotyledons and seedling death were associated either with root and hypocotyl necroses or with girdling of the hypocotyl at the cotyledonary level in the absence of any underground symptoms. Roots affected by watery decay were tapered at the distal end, to which soil adhered.

Prevalence and incidence of disease. All symptoms associated with the damping-off complex occurred in the 4 yr of the surveys. The disease complex was widespread in the Guadalquivir Valley, with dead seedlings occurring in 82% of the fields in 1984 and 94% in 1983. Incidence of dead seedlings within the fields varied slightly from year to year, within a range of 6.6–9.4% (Table 1). Seventy percent of 143 fields had less than 10% dead seedlings, and 11% of the fields had more than 20% dead seedlings.

Although the incidence of dead seedlings in affected fields was rather low (Table 1), the incidence of necrosis affecting root and hypocotyl tissues was much higher. Symptoms occurred in 119 of the 120 fields surveyed in 1980, 1983, and 1984. Thirty percent of the diseased fields had symptoms on 25–50% of the plants, and 50% of the fields had more than 50% of seedlings with symptoms.

Isolation and identification of fungi. *Fusarium* spp., *R. solani, Pythium ultimum, T. basicola*, and *Phytophthora palmivora* MF1 A1, in order of decreasing frequency, were isolated from symptomatic seedlings in fields in 1980–1984. More than one of these fungi was often isolated from individual seedlings, with the association of *Fusarium* spp. and *R. solani* being the most common.

Fusarium spp. were the only fungi isolated from symptomless seedlings. Also, *Fusarium* spp. were the fungi most frequently isolated from any symptom complex except cotyledon collapse (Table 2). Isolation of *R. solani* was most frequent from dead seedlings, and from seedlings affected by severe necrosis of root and/or hypocotyl, and to a lesser extent from seedlings with restricted necrosis of root and hypocotyl (Table 2). In a few cases, binucleate *Rhizoctonia*-like fungi were also recovered from affected tissues. No *Rhizoctonia* spp. were isolated from healthy seedlings or from seedlings affected by watery decay of roots or cotyledon collapse. Of the 37 isolates of *Rhizoctonia* spp. that were studied, 30 were *R. solani* assigned to AG-4 after anastomoses testing, and seven were binucleate *Rhizoctonia*-like fungi.

Pythium ultimum was associated primarily with watery decay of the root, but it was also recovered from roots with extensive necrosis. *Phytophthora palmivora* MF1 A1 and *T. basicola* were isolated from diseased seedlings at very low frequencies. While symptom development was associated with at least one main fungal species, only nonpathogenic miscellaneous fungi (species of *Alternaria, Chaetomium, Penicillium, Stachybotrys, Stemphylium*, etc.) and, in a low percentage, *Fusarium* spp. were recovered from seedlings affected by cotyledon collapse (Table 2).

Pathogenicity and virulence of isolates. All isolates of *R. solani* and two of seven isolates of binucleate *Rhizoctonia*-like fungi were pathogenic to cotton. Isolates of *R. solani* induced preemergence seedling death and restricted necrosis on roots and hypocotyls of emerged seedlings, which often resulted in seedling death. Pathogenic isolates of binucleate *Rhizoctonia*-like fungi induced small lesions in roots of a few inoculated seedlings.

In a preliminary test, virulence of four isolates of *R. solani* AG-4 to Tabladilla 100 varied with inoculum rate. At the highest inoculum rate (17 g/kg of soil), seedling emergence was less than 8% for all four isolates. When inoculum was used at the rate of 1.7 g/kg of soil, seedling emergence ranged from 22 to 81%, but postemergence damping-off was 100% for three isolates and 60% for another. At the lowest inoculum rate tested (0.17 g/kg of soil), seedling emergence was much higher and postemergence damping-off ranged from 8 to 72%. The binucleate *Rhizoctonia*-like fungus used in this test was not pathogenic. Of the 30 isolates of *R. solani* AG-4 evaluated at the rate of 1 g/kg of soil, 23 were highly virulent to Coker 310, with a disease index of 5–6. Six isolates were moderately virulent (disease index 3–5), and one isolate was weakly virulent (disease index 2–3). Five of seven binucleate *Rhizoctonia*-like fungi were not pathogenic, and two were weakly virulent (disease index 2–3).

Isolates of *Pythium ultimum* induced pre- and postemergence damping-off and root necrosis of surviving seedlings of Coker 310 inoculated at all three inoculum rates (Table 3). There was no significant interaction between isolate and inoculum rate, and only the highest inoculum rate resulted in a significant ($P = 0.01$) increase of preemergence damping-off. As a result, values averaged across inoculum rates are used in Table 3. Isolates Pu315 and Pu375 induced the lowest seedling emergence ($P = 0.01$), and isolate Pu375 induced the highest postemergence damping-off (Table 3).

Isolates of *Phytophthora palmivora* MF1 A1 were highly virulent to Coker 310, inducing severe pre- and postemergence damping-off at all three inoculum rates. There was no significant interaction between isolate and inoculum rate. Isolate Pp353 induced preemergence damping-off (85%) at a rate significantly ($P = 0.01$) higher than that for isolate Pp165 (66%) but, based on disease index, both isolates were equally virulent. Both isolates induced nearly 100% postemergence damping-off.

Isolates of *T. basicola* caused severe stunting, hypocotyl necrosis, and root rot but only a low incidence (20%) of postemergence damping-off. Disease indices were not influenced by the inoculation method, although incidence of postemergence damping-off was higher when soil was infested with endoconidia. The two isolates tested were equally virulent to Coker 310. Virulence based on disease indices increased significantly ($P = 0.01$) with inoculum concentration. Mean disease indices of 3.9, 4.3, and 4.5 were recorded for 2×10^2, 2×10^3, and 2×10^4 endoconidia per gram of soil, respectively.

Of the 152 isolates of *Fusarium* spp. tested in preliminary inoculation experiments, 36 induced necrotic lesions on root and/or hypocotyl tissues with an incidence higher than 25%. In further experiments, only five of the 36 isolates were pathogenic to cotton, including two isolates each of *F. oxysporum* and *F. solani* (Mart.) Sacc. and one isolate of *F. equiseti*. The two isolates of *F. oxysporum* induced mild and moderate root necrosis (mean indices 1.9 and 3.3) and postemergence damping-off of 8.2 and 28.6%, respectively. The two isolates of *F. solani* induced mild root necrosis (mean indices 2.2 and 2.5). The isolate of *F. equiseti* induced 35 and 26% pre- and postemergence damping-off, respectively, and severe stunting and root necrosis of emerged seedlings (mean index 3.9).

DISCUSSION

Seedling damping-off was widespread

Table 3. Severity of damping-off caused by *Pythium ultimum* on cotton cultivar Coker 310[x]

Treatment (isolate)	Emergence (%)	Postemergence damping-off (%)	Average disease index[y]
Control	88	0	1.0
Pu315	33[z] a	7 a	2.6 a
Pu373	67 b	1 a	2.5 a
Pu375	48 a	15 b	3.0 a

[x]Seeds were sown in an autoclaved soil mixture infested with isolates grown in a cornmeal-sand mixture at the rates of 4, 10, and 40 g/kg of soil. Values are averages over inoculum rates.

[y]Assessed on a 1–6 scale (1 = no symptom, 6 = dead seedling) 4 wk after sowing.

[z]Values in a column followed by different letters are significantly different according to Student's *t*-test at $P = 0.01$ (emergence) or $P = 0.05$ (postemergence damping-off, average disease index).

and affected cotton severely in southern Spain. Percentage of fields affected and incidence of the disease complex in them varied slightly during the 4 yr of the surveys. The disease is, thus, endemic in the area. Nevertheless, the importance of the seedling disease complex could increase if crop practices, weather conditions, or increase of inoculum of the pathogens involved favor the development of the diseases in the complex. Although measurements of lint yield losses were not made, we consider this disease complex to be of economic importance in southern Spain because its occurrence results in replanting, late crop maturity, and harvest delays (11).

Symptoms observed on seedlings affected by the disease complex in southern Spain are similar to those reported from other countries (1,12,14,16,17). Results from isolations and pathogenicity tests indicate that several genera of fungi are involved as agents of the disease complex. However, the high frequency in which no microorganisms were isolated from affected tissues (Table 2) suggests that abiotic agents could be involved. That is particularly relevant with collapsed cotyledons, from which only nonpathogenic fungi were isolated. Thus, it appears that cotyledonary collapse in southern Spain is different from that described by Minton and Garber (12), which was due to infections of cotyledons before emergence.

R. solani AG-4, *Pythium ultimum, T. basicola*, and *Phytophthora palmivora* were isolated from affected tissues and shown to be pathogenic to cotton under specific controlled conditions. *R. solani* AG-4 is the most important pathogen in the complex in southern Spain, as it is in other areas (1,3,16,17). Most isolates of *R. solani* AG-4 tested were highly virulent to the cotton cultivar currently grown in the area. Although some isolates of binucleate *Rhizoctonia*-like fungi were also recovered from affected tissues, they were mostly not pathogenic to cotton.

Several species of *Pythium* have been reported to be pathogenic to cotton (6,9). However, *P. ultimum* is the only species isolated from necrotic roots of emerged seedlings in southern Spain. The frequency of isolation of *P. ultimum* was low compared to that reported by others (1,14). This does not necessarily imply a reduced importance of *P. ultimum* in the complex, since it might also induce a preemergence damping-off, as indicated by our pathogenicity tests. No efforts were made to determine the etiology of preemergence damping-off observed in the disease surveys.

T. basicola and *Phytophthora palmivora* MF1 A1 appear to play a minor role as pathogens for cotton seedling damping-off under our conditions, as indicated by frequency of isolations (Table 2). However, our isolates of *P. palmivora* MF1 A1 were extremely virulent to Coker 310. Despite the low frequency of isolation, the extreme virulence should not be overlooked. This is the first report of *P. palmivora* MF1 A1 as an etiological component of cotton seedling disease complex.

Results with isolations and pathogenicity tests of *Fusarium* spp. indicate that they must be of minor significance in the etiology of cotton seedling damping-off, as reported by other researchers (1,3,9,12,16). Nevertheless, some isolates of *F. oxysporum, F. solani*, and particularly *F. equiseti* were virulent in pathogenicity tests, which indicates that components pathogenic to cotton exist. Because those species, as well as others, are important in the etiology of the disease complex in some areas (5,14), further studies should be conducted to determine the precise role of *Fusarium* spp. in the etiology of the disease complex in southern Spain.

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