Etiology, Importance, and Distribution of Verticillium Wilt of Cotton in Southern Spain

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ABSTRACT

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Surveys for Verticillium wilt in 142 cotton fields in 1981 to 1983 and 1985 indicated that the disease is widespread in the Guadalquivir Valley, southern Spain, where it occurs in 80.0 to 82.5% of the fields. Verticillium wilt was most prevalent in the upper valley in the 1981 to 1983 surveys and in the lower valley in the 1985 survey. Analyses of dry soil samples collected in spring 1985 by means of an Andersen sampler detected *Verticillium dahliae* propagules in 35.1% of the fields surveyed. Inoculum density in soil was much higher in the lower valley (average 37.1 CFU/g) than in the upper and central areas of the valley (average 4.7 CFU/g). The increasing importance of the disease in the lower valley corresponds to the spread of a defoliating pathotype of *V. dahliae* in this area during the early 1980s. Two groups of isolates, including 100 mild nondefoliating and 90 severe defoliating, were distinguished among 191 isolates by means of morphological, physiological, and pathogenicity tests. The remaining isolate was identified as nondefoliating with intermediate virulence. All defoliating isolates were obtained from the lower valley, while nondefoliating isolates were widespread. Isolates of the defoliating pathotype produced elongated and rounded microsclerotia on water agar, were able to grow on sanguinarine-amended potato dextrose agar, and fluoresced under UV light. Isolates of the nondefoliating pathotype formed only rounded microsclerotia on water agar, were inhibited greatly on sanguinarine-amended PDA, and did not fluoresce under UV light. Furthermore, the defoliating isolates had an optimum temperature for in vitro growth of 24 to 27°C compared with 21 to 24°C for the nondefoliating isolates.

Additional keyword: *Gossypium hirsutum*

About 83,000 ha of upland cotton (*Gossypium hirsutum* L.) are grown annually under irrigation in Andalucía, southern Spain (3). This area, which amounts to about 94% of the total national area for cotton production, is concentrated mainly in the Guadalquivir Valley. In the 1960s, approximately 350,000 ha of cotton were grown in Andalucía. Thereafter, a steady reduction in cotton production has occurred, because of abandonment of dry land cotton, increased production costs, and losses due to pests and diseases.

Verticillium wilt (VW) of cotton, caused by *Verticillium dahliae* Kleb., was first reported in Virginia in 1914 (11), but was not identified as an important disease under field conditions until 1927 in Tennessee (31). VW is now recognized as one of the major problems of irrigated cotton in the U.S. (12) and in most cotton-growing areas of the world (5). In spite of the widespread distribution of the disease, information about yield losses is lacking in most countries. In the U.S., yield losses due to VW for the period 1952 to 1990 were estimated to be 2.2%, with a peak loss of 4.4% in 1961 (5). Losses of lint in some fields in California have exceeded 75% and entire fields have been destroyed in Texas (28). Losses in the former Soviet Union were estimated at 8 to 10% (28), and annual losses of 4% were reported in Syria (20).

VW was first reported in northern Spain in 1954 (2). The disease was first described in Andalucía in 1972 (17), although farmers had observed symptoms of the disease for several years prior to this report. Nevertheless, detailed information on the relative importance of VW of cotton in Andalucía was lacking. Control of the disease is best achieved by tolerant cultivars, the effectiveness of which is overcome by highly virulent strains or high inoculum densities of the pathogen (27,29). This paper presents results of research to determine the incidence and severity of VW on cotton in Andalucía, and on the virulence of isolates and inoculum density of *V. dahliae* from this area. A preliminary report of this work has been published (6).

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MATERIAL AND METHODS

Disease surveys. Systematic surveys for VW were carried out in the cotton-growing area of Andalucía along the Guadalquivir Valley in 1981 to 1983, and 1985. A total of 142 fields, sown mostly to cv. Coker 310, were inspected. Fields were arbitrarily chosen along predetermined routes.

A total of 85 fields were surveyed in 1981 to 1983: 21, 20, and 44 in 1981, 1982 and 1983, respectively, at the time of boll opening. Incidence (percentage) of plants showing foliar symptoms characteristic of VW (28) was assessed in a minimum of three representative groups of 20 consecutive plants, which were arbitrarily chosen in each field. Disease severity was evaluated for each plant on a 0 to 4 scale according to the percentage of foliage affected by chlorotic, necrotic, and wilt symptoms and/or defoliation, in an acropetal progression (0 = no symptoms; 1 = 1 to 33% foliage affected; 2 = 34 to 66% foliage affected; 3 = 67 to 100% foliage affected; 4 = dead plant). Incidence and severity values of foliar symptoms were used to calculate a disease intensity index (D_I), ranging 0 to 100%, according to the following equation (1): $D_I = (I \times S) / M$, where I = incidence of diseased plants (%), S = mean severity of foliar symptoms in diseased plants, and M = maximum severity value (i.e., 4). D_I gives the mean value of disease intensity in a crop at a given time as a percentage of the maximum possible disease intensity.

In 1985, inoculum density of *V. dahliae* in soil of 57 cotton fields was determined in May, 15 to 30 days after planting, with the crop mainly at the cotyledonary stage. Thirty 200-g soil samples were taken from the upper 20 cm of soil from each field with a cylindrical (2.5 × 20 cm) auger, according to a sampling pattern with three

diagonal paths (10) corresponding to an area of ca. 40 × 40 m. Soil samples from each field were bulked and manually mixed, then air dried for 5 to 6 months at 30 to 50% relative humidity and 22 to 25°C. Afterward, each composite sample was homogenized for 20 min in a rotating mill with steel cylinders, and sieved through a 0.8-mm-pore sieve. Five 100-mg aliquots of dry soil from each composite were plated onto a semiselective sodium/polypectate agar medium (SPA) (8) with an Andersen air sampler (Andersen Samplers, Inc., Atlanta) (13). Plates were incubated for 14 days at 23 to 25°C in the dark, then soil residues were removed under tap water and colonies of *V. dahliae* formed on the SPA medium were counted. Inoculum density in each soil sample was estimated by the number of colonies of *V. dahliae* that developed, and expressed as the number of CFU per gram of dry soil. VW in the sampled fields was assessed in September 1985, when the crop was at the stage of the first bolls split. For each field, six groups of 20 consecutive plants were arbitrarily chosen within the same area where soil had been sampled in May. Incidence (percentage) of plants with VW (as indicated by the occurrence of vascular discoloration) was determined by counting the number of plants showing a brown discoloration of the vascular tissue in the stem 4 to 6 cm above the soil level. Additionally, incidence and severity of foliar symptoms (according to the 0 to 4 scale described above) as well as D_I values were determined in 54 of the surveyed fields.

Isolation from affected plants. In 1981 to 1983 and 1985, at the time of VW assessment, stems and petioles of plants affected with a range of symptom severities were sampled from each field for isolation of the pathogen. Tissues were washed under running tap water, then surface disinfested in 0.5% NaOCl for 1 to 2 min and dried between sterile filter paper. Pieces, 5 to 10 mm long, of disinfested tissues were plated onto water agar (WA), water agar amended with aureomycin (WAA) (1 liter of distilled water, 20 g of agar, 30 mg of aureomycin) or potato dextrose agar (PDA). Tissues were incubated at 22 to 24°C in the dark. Isolates of *V. dahliae* were identified according to the taxonomic features of the fungus (32). Monosporic isolates of *V. dahliae* were obtained from colonies that developed in plum extract agar (PEA) (900 ml of distilled water, 20 g of agar, 100 ml of concentrated plum extract, 1 g of yeast extract, 5 g of lactose, pH 5.6 to 6.0). These cultures were stored by covering with liquid paraffin (autoclaved twice at 121°C for 70 min) (E. Merck, Darmstadt, Germany) at 5 to 10°C in the dark.

Characterization of *V. dahliae* isolates. Experiments were carried out to determine differences in virulence among isolates of *V. dahliae* obtained during surveys in 1981 to 1983 and 1985 on several cotton cultivars. Seeds were disinfested in 1% NaOCl for 2.5 min, and sown in 15- or 12-cm-diameter clay or plastic pots, one plant per pot. The potting medium was either a sterilized sand/clay loam/peat mixture (1:1:1, vol/vol/vol; autoclaved twice at 121°C for 90 min) or a nonsterile sand/loam/peat mixture (2:1:1, vol/vol/vol). Plants were grown in a greenhouse with a 12-h photoperiod and fluctuating temperatures that ranged from 19 to 29°C during the light period and from 14 to 20°C during the dark period. Plants were watered as required, and fertilized every 2 weeks with a water soluble fertilizer (20-10-20, N-P-K).

For inoculum, isolates of *V. dahliae* were grown on PDA slants for 7 days at 24°C in the dark. Conidia were suspended in sterile distilled water by gently rubbing the surface of the colony with a sterile bent glass rod. Conidial suspensions were filtered through three to four layers of sterile cheesecloth and their concentration was adjusted to 2.5 to 3 × 10^6 conidia/ml with a hemacytometer. For inoculations, stems were punctured at two opposite points at the base of the first and second internodes, and one 5-µl droplet of conidial inoculum was deposited in each hole (7). Control plants were treated similarly with sterile water. Plants 3 to 4 weeks old and 6 to 7 weeks old were used for inoculations with isolates from 1981 to 1983, and 1985, respectively.

Severity of disease reaction was assessed according to the 0 to 4 scale described above, at 2, 3, 4, and 5 weeks after inoculation. Plant height and fresh weight from the cotyledonary node were determined for plants inoculated with isolates obtained in 1985. Analysis of variance and mean comparisons were performed with data on plant height, fresh weight, and final disease severity.

Virulence to cv. Coker 310 of 18 mass isolates of *V. dahliae* obtained during the 1981 to 1983 disease surveys was tested first under the greenhouse conditions listed. There were 29 replicated pots in a completely randomized design. Differences in virulence among 14 selected mass isolates of the pathogen to cvs. Coker 310, Acala 4-42, Acala SJ-2, Acala SJ-5, and Acala SJC-1 and line PI 70-110 were investigated in a series of five experiments in a growth chamber. The growth chamber was adjusted to a 14-h photoperiod of fluorescent light of 216 to 270 $\mu E \cdot s^{-1} \cdot m^{-2}$. Temperature and relative humidity, respectively, were 21 to 27°C and 50 to 90% during the light period, and 18 to 22°C and 60 to 100% during the dark period. Experiments were designed as randomized complete blocks, with 14 to 20 replicated pots. The series of experiments had some cultivars and isolates in common, so that results could be compared across experiments. All cultivars except Coker 310 were provided by J. E. DeVay (Department of Plant Pathology, University of California, Davis). Seventeen of the 18 isolates were further characterized regarding their microsclerotial morphology and their optimum temperature for in vitro mycelial growth (27). The morphology of microsclerotia was studied in colonies grown on WA in petri plates at 24°C for 21 days. In vitro growth was determined by the diameter of colonies that developed on PDA in petri plates at 20, 24, or 27°C. Data were analyzed by Statistix (Analytical Software, Roseville, MN).

A series of nine experiments was carried out in the greenhouse to determine differences in virulence to cotton Acala SJ-2 and PI 70-110 among 173 monoconidial isolates of *V. dahliae* obtained in 1985. These isolates were collected from 43 of the 57 surveyed fields. The experiments had a randomized complete block design with six replicated pots per isolate/cultivar combination, except for one experiment for which there were five replications. Isolates V4 and V117 of *V. dahliae*, previously characterized as moderate nondefoliating and severe defoliating, respectively, were used as reference isolates. Similarity

Table 1. Incidence and severity of Verticillium wilt of cotton in the Guadalquivir Valley of Andalucía, southern Spain, in 1981 to 1983

Area	Fields sampled	Fields with plants showing foliar symptoms (%)	Incidence of plants with foliar symptoms (%)[x]		Disease severity[y]		Disease intensity index (%)[z]	
			Mean	Range	Mean	Range	Mean	Range
Upper	36	100.0	13.6	0.8 to 75.0	1.9	0.9 to 3.0	6.6	0.4 to 45.0
Central	32	65.6	23.6	0.8 to 66.7	1.8	1.1 to 3.0	11.1	0.4 to 45.0
Lower	17	64.7	24.6	0.8 to 98.3	2.3	0.5 to 3.6	14.7	0.5 to 88.5
Total	85	80.0	18.5	0.8 to 98.3	1.9	0.5 to 3.6	9.3	0.4 to 88.5

[x] Assessed at the time of boll opening in a minimum of three groups of 20 consecutive plants that were arbitrarily chosen in each field.

[y] Assessed on a 0 to 4 scale based upon the percentage of foliage with Verticillium wilt symptoms (0 = no symptoms; 1 = 1 to 33% foliage affected; 2 = 34 to 66% foliage affected; 3 = 67 to 100% foliage affected; 4 = dead plant).

[z] Calculated on a 0 to 100 scale (0 = all plants asymptomatic, 100 = all plants dead) according to the equation $D_I = (I \times S) / M$, where I = incidence of diseased plants (%), S = mean severity of foliar symptoms in diseased plants, and M = maximum severity value (i.e., 4).

among disease reactions for V4 and V117 isolates on cultivars across experiments was tested by analysis of data arranged in a split-plot design in which the experiments were main plots and the isolate/cultivar combinations were subplots (14). Statistical analyses were performed with Statistix.

Microsclerotial morphology on WA and growth on sanguinarine-amended PDA also were determined for the 173 monoconidial isolates. Isolates were grown on WA at 24°C in the dark for 28 to 35 days and the morphology of microsclerotia formed was characterized under the light microscope (27). Isolates were grown on PDA supplemented with sanguinarine nitrate 10^{-3} M at 24°C in the dark for 7 to 14 days and growth response and fluorescence under UV light (360 nm) were determined (22, 27). The optimum temperature for in vitro mycelial growth (27) was investigated in eight selected isolates of diverse geographical origin. These isolates were representative of those identified as defoliating or nondefoliating in the pathogenicity experiments. Isolates SS-4 and T-1, which are representative of nondefoliating and defoliating pathotypes, respectively, from California, (29) were obtained from J. E. DeVay and also used. The 10 isolates were grown on PDA in petri plates at 21, 24, 27, or 30°C in the dark. There were 12 replications (plates) per isolate-temperature combination. Mean colony diameter was determined at 7, 14, 21, and 27 days of incubation, and growth rates (mm/day) were estimated by the slopes of linear regressions of colony diameter over time. For all in vitro experiments, isolates V4 and V117 of *V. dahliae* were included as reference isolates.

RESULTS

Importance and distribution of the disease. Symptoms in affected plants closely resembled those described in the literature for VW of cotton, including foliar chlorosis and necrosis, early plant senescence, stunting, defoliation, and vascular discoloration (5,28). Symptoms were most conspicuous by early boll development, in early September.

The incidence and severity of the disease were fairly similar among the 3 years of the 1981 to 1983 surveys. Frequency of affected fields between years varied from 76 to 82% of the inspected fields, with an average incidence of plants with foliar symptoms of 18.0 to 19.3%, and disease severity between 1.4 and 2.1. VW was extensively distributed throughout the Guadalquivir Valley, although its prevalence along the valley was uneven, with more fields being affected in the upper valley (100.0%) than in the central (65.6%) and lower (64.7%) valley (Table 1). However, mean incidence of foliar symptoms and disease intensity were higher at the central and lower valley than the upper valley, and plants affected in the lower valley had the highest average disease severity. Mean incidence of foliar symptoms ranged from 13.6 to 24.6% for three areas of the valley, with an average value of 18.5% (Table 1). Nevertheless, 3 of the 11 affected fields in the lower valley had a disease incidence higher than 50%.

In 1985, 82.5% of the surveyed fields had plants with vascular discoloration, with an average incidence of 22.4% of the plants but only 74.1% of the fields had plants with foliar symptoms (Table 2). Average incidence of foliar symptoms and disease intensity in affected fields were 20.4 and 10.8%, respectively (Table 2), slightly higher than in the 1981 to 1983 surveys. All plants with foliar symptoms also showed vascular discoloration. Frequency of fields with symptomatic plants (vascular discoloration or foliar symptoms) was higher in the lower valley than in the central and upper valley (Table 2). Mean incidence of plants with vascular discoloration or foliar symptoms, disease severity, and disease intensity were also higher in the lower valley than in the other cotton areas surveyed. Percentage of affected fields was similar in the upper and central valley, but mean incidence of vascular discoloration and foliar symptoms, as well as disease intensity, were higher in the upper valley (Table 2). In 1985, 75% of all affected fields had incidences of foliar symptoms below 25%, but incidences higher than 50% occurred in 5 fields (29.4%) in the lower valley.

Twelve fields where cotton had been grown repeatedly and seven fields where cotton had not been cropped frequently were compared for disease occurrence. VW occurred in all 12 fields with intense cotton cultivation but only in two of the seven without intense cotton cultivation. Incidence of vascular discoloration and foliar symptoms in the 12 fields ranged from 3.3 to 98.3% (average 47.2%) and 0.8 to 97.5% (average 41.6%), respectively. In comparison, in the two affected fields without intense cotton cultivation the incidence of vascular discoloration was 0.8 and 15.0%, respectively, and foliar symptoms only occurred in the field with more vascular discoloration, with an incidence of 14.2%.

Inoculum density of *V. dahliae* in soil. *V. dahliae* was detected in 35.1% of the field soils sampled in 1985, with a mean inoculum density of 14.4 CFU/g (Table 3). The pathogen was detected in a similar frequency of fields in the upper, central, and lower areas of the Guadalquivir Valley, but the mean inoculum density was much higher (37.1 CFU/g) in the lower valley than in the central (3.4 CFU/g) and upper (6.4 CFU/g) valley (Table 3). Most (95%) of the fields that were affected by VW but did not yield *V. dahliae* from soil had a low incidence (<25%) of foliar symptoms. On the other hand, *V. dahliae* was detected in eight of the 12 fields with a history of intense cotton, with a mean inoculum density of 29.2 CFU/g (range 2.0 to 132.0 CFU/g), whereas the pathogen was recovered from only one (6.5 CFU/g) of the seven fields without intense cotton cultivation.

Virulence of isolates. Results with isolates from the 1981 to 1983 surveys were consistent over experiments. Disease reaction varied with isolates of the pathogen and tolerance of cotton cultivars, but there

Table 2. Incidence and severity of Verticillium wilt of cotton in the Guadalquivir Valley of Andalucía, southern Spain, in 1985

	Assessment of vascular discoloration				Assessment of foliar symptoms							
		Fields with plants showing vascular discoloration (%)	Incidence of plants with vascular discoloration (%)[x]			Fields with plants showing foliar symptoms (%)	Incidence of plants with foliar symptoms (%)[x]		Disease severity[y]		Disease intensity index (%)[z]	
Area	Fields sampled		Mean	Range	Fields sampled		Mean	Range	Mean	Range	Mean	Range
Upper	16	75.0	21.0	0.8 to 79.3	15	60.0	20.8	1.7 to 74.2	1.4	1.0 to 2.0	8.9	0.4 to 37.3
Central	23	78.3	16.1	0.8 to 73.3	21	66.7	12.2	0.8 to 44.2	1.7	0.5 to 2.8	5.6	0.1 to 15.5
Lower	18	94.4	30.1	0.8 to 98.3	18	94.4	27.0	0.8 to 97.5	2.2	1.0 to 3.6	16.0	0.2 to 61.2
Total	57	82.5	22.4	0.8 to 98.3	54	74.1	20.4	0.8 to 97.5	1.9	0.5 to 3.6	10.8	0.1 to 61.2

[x] Assessed at the time of boll opening in six groups of 20 consecutive plants that were arbitrarily chosen in each field.

[y] Assessed on a 0 to 4 scale based upon the percentage of foliage with Verticillium wilt symptoms (0 = no symptoms; 1 = 1 to 33% foliage affected; 2 = 34 to 66% foliage affected; 3 = 67 to 100% foliage affected; 4 = dead plant).

[z] Calculated on a 0 to 100 scale (0 = all plants asymptomatic, 100 = all plants dead) according to the equation $D_I = (I \times S) / M$, where I = incidence of diseased plants (%), S = mean severity of foliar symptoms in diseased plants, and M = maximum severity value (i.e., 4).

was not statistically significant isolate × cultivar interaction. Most isolates, represented by isolate V4, induced mild foliar symptoms (interveinal chlorosis followed by necrosis and wilting) restricted to the lower portion of the plant, but caused neither stunting nor defoliation of the plant (Table 4). A second group of isolates, represented by V96, V101, V102, and V103, induced severe foliar symptoms, defoliation, stunting, and death of the plants (Table 4). Isolate V99 induced severe foliar symptoms including stunting, but no defoliation, and death of the plants (Table 4). Isolates inducing mild or severe foliar symptoms without defoliation formed only round, globular microsclerotia in WA, and grew faster at 24 than at 27°C. Defoliating isolates formed both round and elongated microsclerotia in WA, and grew faster at 27 than at 24°C. All defoliating isolates were obtained from the lower valley, while mildly or highly virulent, but nondefoliating, isolates were obtained from the lower, central, and upper valley.

Isolates from the 1985 surveys were tested with isolates V4 and V117 as standards. Isolate V4 induced mild foliar symptoms in leaves up to mid stem of PI 70-110 but usually affected only lower leaves on the VW-tolerant Acala SJ-2. In all cases, this isolate caused neither defoliation nor plant death. Symptoms induced by isolate V117 included defoliation, stunting, and death, and developed earlier and more severely than those induced by V4. Symptoms progressed faster and were more severe on PI 70-110 than on Acala SJ-2. Some differences occurred across experiments in the severity of disease reactions and reduction in plant height and fresh weight induced by isolates V4 and V117, which could be related to variation in environmental conditions. However, there was consistency among experiments with regard to defoliating and nondefoliating reactions induced by isolates V117 and V4, respectively. Also, these two isolates differed significantly in virulence on the two cotton cultivars in all experiments.

Isolates of *V. dahliae* obtained in 1985 were differentiated into two groups by the disease reaction induced on the cotton cultivars. One group consisted of 87 isolates that induced disease reactions similar to those described for the mildly virulent isolate V4. These isolates formed only globular microsclerotia on WA, and their radial growth on sanguinarine-amended PDA was inhibited, though abundant aerial mycelium formed on the transferred PDA disks. Also, colonies of these isolates did not fluoresce when exposed to UV light. The other group consisted of 86 isolates that induced severe disease reactions similar to those induced by isolate V117. These isolates formed both elongated and rounded microsclerotia on WA, their growth was not inhibited on sanguinarine-amended PDA, and their colonies emitted a shining blue fluorescence when exposed to UV light. Growth of isolate V99 (highly virulent but nondefoliating) on sanguinarine-amended PDA was similar to that described for the group of nondefoliating, mildly virulent isolates.

In each experiment, the severity of foliar symptoms and reduction in plant height and fresh weight caused by isolates of the defoliating group were, on the average, larger than those caused by nondefoliating isolates. The differences were statistically significant in all experiments except one. Inoculation with nondefoliating isolates compared with controls reduced significantly ($P < 0.05$) fresh weight of both cultivars in all experiments except for Acala SJ-2 in two experiments. On the other hand, differences in height between plants inoculated with nondefoliating isolates and controls were significant only in two experiments when the average for the two cultivars was considered, and in another two experiments only for PI 70-110. A summary of the mean reaction of PI 70-110 and Acala SJ-2 to inoculation with defoliating and nondefoliating isolates of *V. dahliae* in the nine experiments conducted is given in Table 5.

Defoliating and nondefoliating isolates differed in their growth rate on PDA at different temperatures. For the nondefoliating isolates, the optimum temperature for mycelial growth, as indicated by the growth rate, was 21 to 24°C; mycelial growth was reduced at 27°C and very limited at 30°C (Fig. 1A). Neither conidia nor microsclerotia formed at 30°C. In contrast, optimum growth temperature of defoliating isolates was 24 to 27°C (Fig. 1B); at 30°C, fungal

Table 3. Inoculum density of *Verticillium dahliae* in soils of cotton fields at the Guadalquivir Valley of Andalucía, southern Spain, in 1985

Area	Fields sampled[y]	Fields where *V. dahliae* was detected in the soil samples (%)	Inoculum density (CFU per g of dry soil)[z] Mean	Range
Upper	16	37.5	6.4	2.0 to 10.0
Central	23	34.8	3.4	2.0 to 6.5
Lower	18	33.3	37.1	2.0 to 132.0
Total	57	35.1	14.4	2.0 to 132.0

[y] Samplings were done in May, 15 to 30 days after sowing. Thirty 200-g soil samples were taken from the upper 20 cm of soil from each field along three diagonal paths.
[z] Determined according to the method of DeVay et al (13) with 5 100-mg aliquots from soil samples.

Table 4. Severity of disease reaction in cotton cultivars inoculated with isolates of *Verticillium dahliae* obtained in 1981 to 1983[y]

Isolate	Cultivar[z] Acala SJC-1	Acala SJ-2	Acala SJ-5	PI 70-110	Mean
V4	1.2	1.2	1.1	1.8	1.3 a
V99	2.9	2.4	2.7	3.3	2.8 b
V96	3.6	3.4	3.7	3.9	3.7 c
V101	3.7	3.9	3.5	3.9	3.8 c
V102	3.2	3.4	3.5	3.9	3.7 c
V103	3.7	3.4	3.5	4.0	3.7 c

[y] Disease severity assessed on a 0 to 4 scale based upon the percentage of foliage with Verticillium wilt symptoms (0 = no symptoms; 1 = 1 to 33% foliage affected; 2 = 34 to 66% foliage affected; 3 = 67 to 100% foliage affected; 4 = dead plant) 5 weeks after inoculation. Plants were inoculated by injecting a droplet of conidial suspension (2.5 to 3.0 × 10^6 conidia/ml) at each of two opposite points of the stem.
[z] Each value is the mean of 29 replications. The isolate × cultivar interaction was not statistically significant. Means followed by the same letter are not statistically different ($P = 0.05$) according to Fisher's protected least significant difference test.

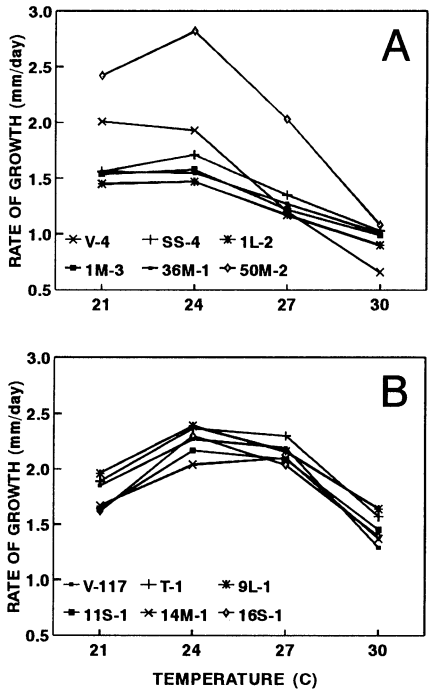

Fig. 1. Growth rate of **(A)** nondefoliating, and **(B)** defoliating isolates of *Verticillium dahliae* on potato dextrose agar at different temperatures in the dark.

growth was not as reduced as for nondefoliating isolates (Fig. 1B) and microsclerotia and conidia were formed. Thus, at 30°C the growth rate of defoliating isolates was always significantly higher than that of the nondefoliating isolates, whereas at 21°C there was an overlapping among isolates of the two pathotypes.

In 1985, nondefoliating isolates were widespread along the Guadalquivir Valley whereas defoliating isolates were found only in 16 of the 43 fields sampled for isolate characterization, all of which were located in the lower valley.

DISCUSSION

VW of cotton is widespread in the Guadalquivir Valley of southern Spain. Mean incidences of plants with vascular discoloration or with foliar symptoms and the mean disease intensity index were lower than 30% during the 4 years of surveys. Nevertheless, the disease was very severe in some fields, which had 90% or more affected plants. Severe disease, including defoliation and death of plants, can occur before the appearance of the first flower buds, by the end of June, or even at the seedling stage, in mid-May.

The severity of VW in the lower Guadalquivir Valley increased steadily over the years of the surveys. Fields in this area were the most severely affected, as shown by their higher values for all disease assessment measurements. Percentages of affected fields in the central and upper valley were similar in 1985, but the mean incidences of foliar symptoms and disease intensity values were higher, and disease severity was lower, in the upper valley. The percentage of fields with plants having symptoms was greater than that of fields in which *V. dahliae* could be detected in soil. Thus, low inoculum densities of the pathogen were not always detected by the method of inoculum quantification. In 1985, the percentage of fields where propagules of *V. dahliae* were detected was similar for the three areas of the valley. However, mean inoculum density in the lower valley was higher than that in the two other areas, and the central valley showed the lowest density. In the lower valley prior to 1985, cotton was grown repeatedly. Thus, high inoculum densities in this area probably were due to a build-up of *V. dahliae* populations in soil following a susceptible crop (4,9,16,25), and to the long survival of microsclerotia of this fungus in soil (15,16,30,34). In the 1985 disease survey, levels of inoculum density and disease were higher in fields where cotton was grown repeatedly than in those where cotton was grown occasionally, which agrees with the association between high levels of *Verticillium* propagules in fields cropped continuously to cotton (16).

Isolates of *V. dahliae* from cotton in southern Spain can be characterized into two groups: (i) cotton-nondefoliating isolates that induce foliar symptoms of mild severity and reduce plant weight and, to a lesser extent, height of infected plants under favorable environmental conditions; and (ii) cotton-defoliating isolates that cause severe foliar symptoms, defoliation and death of infected plants, and drastic reduction in plant weight and height. In addition, a single isolate (V99) of intermediate virulence was identified that caused stunting and death of cotton plants but not defoliation. Because only one isolate of this type was found among the large number of isolates tested, this type must occur at a very low frequency in Andalucía.

Nondefoliating, mildly or highly virulent isolates of *V. dahliae* formed only round microslerotia in WA, did not grow on sanguinarine-amended PDA, and had a higher growth rate on PDA at 21 to 24°C than at 27°C, with little growth at 30°C. By contrast, the defoliating isolates formed both elongated and round microsclerotia on WA, grew well on sanguinarine-amended PDA, and had higher growth rates at 24 to 27°C than at 21°C, with moderate growth at 30°C. Since nondefoliating isolates of mild or intermediate virulence consistently have similar morphological and physiological features that are different from those of defoliating isolates, these features in unknown isolates of *V. dahliae* should be useful to identify pathotypes. The two main groups of isolates of *V. dahliae* from cotton in Andalucía seem to be similar to isolates SS-4 and T-1, which are representative of nondefoliating and defoliating pathotypes, respectively, described in the U.S. (22,27,29).

Since the cotton-defoliating pathotype of *V. dahliae* has been recorded only in the Americas and Asia (5,19,21,26,29), its discovery in Spain allows for speculation about its origin in Europe. It may be a variant of the native population, or it may have been introduced from outside Spain. The former hypothesis is not likely because the defoliating isolates belong to a unique vegetative compatibility group (VCG) and vary in several characteristics from nondefoliating isolates (18,23,24, 33). Preliminary results from studies on vegetative compatibility indicate that defoliating and nondefoliating isolates of *V. dahliae* from Spain belong to different VCGs (C. Pérez-Lara, E. Pérez-Artés, and R. M. Jiménez-Díaz, unpublished data). Molecular characterization of defoliating and nondefoliating isolates from southern Spain is in progress.

The increase of the defoliating pathotype of *V. dahliae* in the lower Guadalquivir Valley from a low frequency in 1981 to 1983 to almost 90% of the cotton fields in 1985 shows that this pathotype spreads very quickly. This fact, and the extreme virulence of defoliating isolates to cotton cultivars susceptible, tolerant, or resistant to the nondefoliating pathotype (29), may explain the increase in the importance of VW in the lower valley from the 1981 to 1983 period to 1985. Because of the threat of the defoliating pathotype of *V. dahliae* mainly to cotton and olive crops, thorough knowledge of the distribution of pathotypes of *V. dahliae* in Andalucía is essential to establish efficient strategies for control of VW.

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Table 5. Disease reaction and growth in cotton cultivars inoculated with isolates of *Verticillium dahliae* obtained in 1985[x]

Pathotype	No. of isolates	Cultivar	Severity[y]		Height (%)[z]		Fresh weight (%)[z]	
			Mean	Range	Mean	Range	Mean	Range
Nondefoliating	87	PI 70-110	1.9	1.3 to 2.8	86.1	56.5 to 115.8	58.9	26.9 to 98.1
		Acala SJ-2	1.5	1.0 to 2.2	90.5	53.9 to 133.3	74.1	39.4 to 107.3
Defoliating	86	PI 70-110	3.5	2.6 to 4.0	57.0	26.8 to 83.1	17.1	2.5 to 46.7
		Acala SJ-2	2.6	1.6 to 3.8	68.5	33.3 to 108.9	36.7	8.8 to 77.6

[x] Results of nine experiments with six replications per isolate/cultivar combination, except for one experiment with five replications, 5 weeks after inoculation. Plants were inoculated by injecting a 5-µl droplet of conidial suspension (3.0×10^6 conidia/ml) at each of two opposite points located on the base of the first and second internodes, and then were incubated in the greenhouse.

[y] Assessed on a 0 to 4 scale based upon the percentage of foliage with Verticillium wilt symptoms (0 = no symptoms; 1 = 1 to 33% foliage affected; 2 = 34 to 66% foliage affected; 3 = 67 to 100% foliage affected; 4 = dead plant) 5 weeks after inoculacion.

[z] Expressed as a percentage of the respective values in the control, noninoculated plants.

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