



Plant Pathology Journal

ISSN 1812-5387

science
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Distribution of Verticillium Wilt in Cotton Areas of Southern Turkey

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Abstract: A survey was conducted to determine incidence, prevalence and inoculum density of *Verticillium dahliae*, the causal agent of Verticillium Wilt (VW) of cotton, in southern Turkey. Survey of VW in 151 cotton fields from 30 locations of Hatay, Kahramanmaraş, Adana, Mersin and Osmaniye provinces in 2000 and 2001 indicated that the disease occurred in 37,1% of the fields. The mean disease incidence of plants with vascular discoloration was 31.2%, the mean severity of the disease was 0.9 ranging from 0.1 to 3.4 (according to 0 to 4 scale) in the region. Analyses of soil samples by wet sieving method revealed that the mean inoculum density ranged from 7 to 28.4 microsclerotia (ms) g⁻¹ of soil in the region.

Key words: Cotton, *Verticillium dahliae*, prevalence, inoculum density

INTRODUCTION

Verticillium Wilt (VW) disease of cotton (*Gossypium hirsutum* L.), caused by soilborne fungal vascular-wilt pathogen *Verticillium dahliae* Kleb., is the most serious disease of cotton worldwide. The disease has become a major constraint in Turkey's cotton production industry^[1,2] as in most major cotton-producing areas of the world^[3].

V. dahliae is widely distributed in the eastern Mediterranean Region Turkey and causes extensive losses on cotton lint yield. This region accounts for ca. 20% of the national upland cotton (*Gossypium hirsutum* L.) production of 917, 000 ton. Adana, Mersin, Kahramanmaraş, Hatay and Osmaniye provinces, the main cotton-producing areas of the region, have the largest sowing area with approx. 138, 000 ha, lint producing 130 kg da⁻¹^[4]. In recent years, despite favorable climatic and edaphic conditions in the region, there has been a reduction in cotton production due to VW. The disease has been increasing in the region due to unplanned crop rotation, cotton monoculture and excessive nitrogen applications.

Since cotton is a crop with high economic importance in the region, it is useful to study and know the epidemiological characteristics of the disease such as disease prevalence, incidence and severity. Moreover, knowing about these kinds of disease parameters and the density of microsclerotia (ms) in soil is important for disease prediction and for applying the control measures. Because the disease can be effectively controlled using

tolerant cultivars, crop rotation, solarization, systemic fungicides, induction of phytoalexins gossypol and principles of integrated pest management^[2,3,5-7].

This study was conducted to determine the prevalence, incidence and severity of VW on cotton in the Eastern Mediterranean Region of Turkey and to determine assay soil inoculum level of *V. dahliae* in cotton areas surveyed

MATERIALS AND METHODS

Disease survey: Commercial cotton fields in the eastern Mediterranean Region of Turkey were surveyed for the incidence and prevalence of VW between the June flowering stage and October harvesting stage in 2000 and 2001. A total of 151 fields in 5 provinces were inspected. Each field was surveyed in an X pattern by walking diagonally across the field. Disease incidence in each field was calculated by counting the total numbers of healthy and diseased plants in 3 to 10 groups of 20 consecutive plants (arbitrarily chosen) based on sizes of fields. Prevalence of the disease was calculated using the percentage of fields with plants exhibiting disease symptoms. Disease severity was evaluated for each plant on a scale of 0 to 4 (0 = no vascular discoloration, 1 = 1 to 25% vascular area discoloured, 2 = 26 to 50% vascular area discoloured, 3 = 51 to 75% vascular area discoloured and 4 = 76 to 100% vascular area discoloured with or without foliar wilting according to the percentage of brown flecking or discoloration of the stem^[8]). Disease Intensity Index was calculated on a 0 to 100 scale

(0 represents all plants asymptomatic, 100 represents all dead plants) according to the equation $D_i = (I \times S)/M$. Where, I is incidence of diseased plants (%), S is mean severity of foliar symptoms in diseased plants and M is maximum severity value i.e. 4.

Within infected field sites, soil samples were collected at a maximum depth of 20 cm to estimate inoculum density of *V. dahliae*. Plants exhibiting symptoms of VW were arbitrarily collected at each site to confirm the causal agent of the wilt symptoms. Incidence, severity and intensity index of the disease and standard mean error were statistically analyzed using SPSS software (SPSS, Inc., LEAD technologies, Chicago, IL).

Isolation of *V. dahliae* from affected plants: Isolates of *V. dahliae* were collected from diseased cotton plants. For isolations from infected plants, root, stem and petiole tissues exhibiting vascular discoloration were rinsed thoroughly in tap water and air-dried for 5 to 10 min. Infected tissues were aseptically cut to dimensions of approx. 5 to 10 mm long and surface-disinfested in 0.525% NaOCl solution for 2 min, rinsed twice in sterile distilled water and dried between sterile filter papers. Disinfested tissues were plated on potato dextrose agar (PDA, Difco) and ethanol agar (20 g of agar, 6 mL of 95% ethanol, 1 L of distilled water) amended with streptomycin sulphate at 100 µg mL⁻¹ to inhibit bacterial growth. Petri plates were incubated at 23 to 24°C in the dark for 5 to 7 days. After incubation, hyphal plugs of each growing isolate colony were transferred to petri dishes (6 cm diameter) of PDA amended with the same concentrations of antibiotics and incubated in the dark at 23°C for 10 days. Colonies forming microclerotia (ms) were identified as *V. dahliae* according to the taxonomic features of the fungus via microscopic examination. Single-spore isolates of *V. dahliae* were obtained from water agar medium amended with 100 µL mL⁻¹ streptomycin sulphate and maintained in vials containing PDA at 4°C.

Assays of inoculum density in soil samples: Inoculum density of *V. dahliae* was assessed from (200 g) soil samples taken at 20 cm depths from 23 cotton fields substantiated for disease incidence and severity. Three, five and seven soil samples were taken from 1.0, 1.0 to 5.0 and 5.0 to 10.0 ha fields, respectively, mixed, air-dried for 1 to 2 weeks at 25°C and passed through a 2 mm screen. Inoculum density of *V. dahliae* in soil samples were detected by wet sieving methods described by Ashworth *et al.*^[9] and Huisman and Ashworth^[10]. Soil samples were wet sieved through a 125 µm sieve nested in 38 µm sieve. Samples were gently sieved with running tap-water for 1 to 2 min. Residues were collected

separately from each sieve in about 10 mL of water by rinsing them off into small beakers. Suspended samples were poured evenly over 10 plates of semi selective sodium polypectate medium (4.0 g of NaNO₃, 1.0 g KCl, 1.0 g MgSO₄.H₂O, 0.02 g FeSO₄, 10 g of polygalacturonic acid, 40 g of agar, 4 mL of Tergitol NP-9, 1 L of distilled water) amended with 100 µL mL⁻¹ streptomycin sulphate. Beaker was rinsed with water to transport trace residues to sample plates. Individual plates were gently tapped and swirled to distribute soil residues evenly. Plates were air-dried 10 to 30 min to remove free water. Plates were incubated 12 to 14 days at 22 to 24°C. Soil residues were removed from plates by holding each plate in a bucket of water and gently rubbing the agar surface with fingers. *Verticillium* colonies were quantified under a dissecting microscope. Total *Verticillium* colony populations from both sieves (20 plates) were combined to determine the overall population of *Verticillium* propagules in the original 10 g soil sample. The mean number of ms g⁻¹ soil and the standard error of the mean were determined using SPSS statistical software (SPSS, Inc., LEAD technologies, Chicago, IL).

RESULTS AND DISCUSSION

During the surveys, epinasty, chlorosis and necrosis on the leaf, defoliation, stunting and light to dark brown vascular discoloration in the main stem, root and branches of diseased cotton plants were observed in the growing season. By late August, overall disease symptoms appeared to be in final stages. VW of cotton was found in 31 and 26 fields in 2000 and 2001 in five provinces, in which 151 fields were sampled, in the region surveyed, respectively. VW of cotton was widespread in Hatay and Kahramanmaraş provinces, with the mean disease incidence being 33.1 and 20.3%, respectively; the prevalence of wilt was 70.2 and 44.4%, respectively in surveyed fields. Intensive cotton cultivation or short-term crop rotations are in the scene to distribute the disease at different levels in these provinces. Prevalence of VW was lowest in Mersin (11.1%) and Osmaniye (18.8%) provinces, with the mean disease incidence 13.7 and 15.3%, respectively (Table 1). The reasons for these sequences may be the low inoculum density of the pathogen in these provinces or appropriateness of the application of principles of field management and the intensity of agricultural activities in the cotton fields in the region. Disease incidence (ranged from 0.9 to 95.7), disease severity (ranged from 0.1 to 3.4) and disease intensity (ranged from 0.1 to 81.4) values were also higher in Hatay, Kahramanmaraş and Adana provinces than in the other two provinces surveyed (Table 1). This may

Table 1: Mean prevalence, incidence and severity of *Verticillium* wilt of cotton in the eastern Mediterranean region, southern Turkey, in 2000 to 2001

Location	Fields sampled	Disease prevalence (%) ^a	Disease incidence (%) ^b		Disease severity ^c		Disease intensity index (%) ^d	
			Mean	Range	Mean	Range	Mean	Range
Hatay	47	70.2	33.1	1.8-95.7	0.9	0.1-3.4	11.8	0.1-81.4
K. Maras	9	44.4	20.3	9.2-34.8	1.1	0.6-1.6	6.3	1.4-14.0
Adana	70	21.4	34.3	10.4-91.3	0.9	0.2-2.9	11.2	0.6-66.9
Osmaniye	16	18.8	15.3	7.2-19.5	0.3	0.1-0.6	1.6	0.1-2.9
Mersin	9	11.1	13.7	0.9-16.8	0.2	0.1-0.3	0.6	0.2-0.9
Total	151	37.1	31.2	0.9-95.7	0.9	0.1-3.4	10.5	0.1-81.4

^a Prevalence of the disease was calculated using the percentage of fields with plants exhibiting disease symptoms^b Disease incidence in each field was calculated by counting the total numbers of healthy and diseased plants in 3 to 10 groups of 20 consecutive plants (chosen arbitrarily) based on sizes of fields^c Disease severity was evaluated for each plant on a scale of 0 to 4 (0 = no vascular discoloration, 1 = 1 to 25% vascular area discoloured, 2 = 26 to 50% vascular area discoloured, 3 = 51 to 75% vascular area discoloured and 4 = 76 to 100% vascular area discoloured with or without foliar wilting according to the percentage of brown flecking or discoloration of the stem)^d Disease Intensity Index was 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)Table 2: The mean inoculum density of *Verticillium dahliae* in soils of cotton fields surveyed in the eastern Mediterranean region of Turkey, southern Turkey, in 2000 to 2001

Location	Fields where <i>V. dahliae</i> was detected in the soil samples (%)	Inoculum density [*] (microsclerotia g ⁻¹ soil)	
		Mean±SE	Range
Hatay	36.4	14.5±0.7	7.0-20.6
Kahramanmaraş	34.0	12.7±0.6	10.2-16.4
Adana	30.2	18.0±0.9	8.4-28.4
Osmaniye	25.3	14.2±0.7	13.0-16.6
Mersin	10.0	12.8±1.9	8.0-18.3

^{*} Inoculum density in soil samples collected from each field during harvest in 2000 to 2001 was assessed using wet sieving method. 200 g soil sample was taken from the upper 20 cm of soil from each field in an x pattern by walking diagonally across the field

mean the pathogen commonly exist in the regional cotton belt and also largely resulting in the disease.

Typical cultures of *V. dahliae* were isolated from representative diseased tissues of cotton plants with external symptoms from all diseased fields surveyed. The colors of colonies of *V. dahliae* were first white, later converting to black with microsclerotial production. Conidiophores were erect, septate, branched and hyaline. Conidia were unicellular, ellipsoid and hyaline about 3 to 5x1.2 to 3.1 µm diameter. Based on the properties of conidia, conidiophores and presence of ms, the fungus was identified as *V. dahliae*. Therefore, the accuracy of the survey results was confirmed by the isolation of the fungi from all fields recorded as pathogen present.

V. dahliae was detected in all representative fields showing symptoms of VW. The mean inoculum density ranging from 7.0 to 28.4 ms g⁻¹ soil was much higher in Adana province (18.0 ms g⁻¹ soil) ranging from 8.4 to 28.4 than the one in Hatay province (14.5 ms g⁻¹ soil) ranging from 7.0 to 20.6 ms g⁻¹ soil due to the history of intense cotton (Table 2). The lower percentage of pathogen presence in the Hatay soils may be due to the fact that the variability is very large in the inoculum density. The variability of the inoculum density for the rest of the provinces was smaller than these two provinces. The lowest inoculum density was found in Kahramanmaraş

having fields without intense cotton cultivation, with 12.7 ms g⁻¹ soil among commercial cotton fields surveyed in the region. There is significant (p<0.05) relation between the mean inoculum density values and the disease severity values, according to the correlation analyses. This level of correlation may suggest that the disease severity levels should be used collectively with the mean inoculum density values in the diagnosis of the problem in the region. These findings were in agreement with the study of El-Zik^[11] reporting that an increase in inoculum density from 5 to 60 ms g⁻¹ soil resulted in increase in the percentage of infected plants from 15 to 95%, respectively. Inoculum density values in the present study were not similar to previous study of Bicici and Kurt^[12] who reported that inoculum assessment of *V. dahliae* in soil samples taken from the region showed generally more than 100 ms g⁻¹ soil. These different results may be related to assay methods of inoculum density in soil. Besides, the inoculum density in the fields sampled may have been overestimated because VW pathogen is not uniformly distributed in infested fields.

Consequently, the present data from an exhaustive survey of cotton production areas in Southern Turkey, reporting the prevalence and severity of VW in affected provinces and the corresponding inoculum density of *V. dahliae* in soils reveals that VW remains a threat to cotton production in the region. In addition, VW severity on cotton is related to the inoculum density of *V. dahliae* in soil at planting. Results from this study should be taken into consideration in establishing efficient strategies such as crop rotation based on the inoculum density associated with the management option for control of VW.

ACKNOWLEDGMENTS

This research was supported by the Scientific and Technical Research Council of Turkey (TUBITAK) and Research Fund of Cukurova University, Adana.

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