Etiology, Incidence and Prevelance of Cotton Wilt Disease and Strains of Wilt Pathogen in Cukurova

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ABSTRACT

Wilt disease surveys in 164 cotton fields of Cukurova indicated that the disease was present in 11 fields in 1994 and 12 in 1995. Verticillium dahliae Kleb. was isolated from diseased plants. Cultural and morphological characters indicated that the fungus produced erect, verticillate conidiophores and ellipsoid, one-celled, 3-5x1.2-3.1μm conidia and black microsclerotia. Disease prevalence in 1994 was 33%, 21%, 20% and 11% in Yuregir, Ceyhan, Imamoglu and Karatas counties, respectively. It was 66%, 50 %, 30%, 25%, 14% in Osmaniye, Kozan, Imamoglu, Yuregir, Ceyhan, respectively, in 1995. The mean highest incidence of wilt in Yuregir was 30% and 28.4% in 1994 and 1995, respectively. Microsclerotia assessments in soil samples by soil dilution with modified soil extract agar showed that inoculum density was lowest in Osm2 soil sample (48.8 microsclerotia /g soil) and highest in Koz3 sample (195.4 microsclerotia/g soil). The mean inoculum density of V. dahliae in infested fields was 129.4 microsclerotia/g soil. 23 V. dahliae isolates from diseased cotton plants in the region were virulent to susceptible eggplant cultivar, Pala. Differentiation of V. dahliae strains was carried out with Acala 1517/V, Deltapine 15/21, Giza 75 cotton cultivars and optimum temperature for growth of isolates in vitro. As a result, 12 isolates were SS-4 and 11 isolates were T-1. T-1 isolates caused epinasty, defoliation and death in different cultivars and optimum temperature for their growth was 27°C. SS-4 isolates showed moderate wilting and non-defoliation with an optimum temperature for growth was 24°C.

Introduction

About 750 000 ha of Upland cotton (*Gossypium hirsutum*) are grown annually in Turkey. One third of the total area for cotton production is concentrated in the Cukurova region. Adana, the main cotton growing area of Cukurova, has the highest sowing area with 121 000 ha and 250 kg/da production (Anon., 1997). Despite favourable climatic and edaphic conditions for cotton in Cukurova, a reduction in cotton production has occurred in recent years due to disease and pest problems, high input costs and unstable prices. The incidence of diseases and pests is the most important factor. Wilt disease has been increasing in Cukurova region due to unplanned crop rotation, cotton monoculture and excessive irrigation and nitrogen applications. Diseases control is difficult because the causal agents are soil-borne and soil sterilization is impractical and expensive. Infected plants in the fields expressed reduction in plant growth, epinasty, dwarfing and chlorosis between the main veins and along the margins on leaves, leading to defoliation and light to dark brown vascular discoloration in the main stem and branches.

The main objective of this study was to determine the causal agent of the wilt disease of cotton, epidemiological characteristics such as disease incidence and severity, inoculum density in cotton field soils and strains of the pathogen in Adana.

Material and Methods

Surveys. Survey studies of the disease in Adana were carried out between the flowering stage in June and harvesting stage in October 1994 to 1995. A total of 164 fields (ca.5000 da) in 11 counties were inspected and the incidence of wilted plants and disease prevalence were determined. Observations were also recorded of cotton cultivars, fertilizers use, cropping history, drainage, levelled and unlevelled conditions and weed species growing in cotton fields.

Isolation from affected plants. Root and stem tissues and petioles were washed under running tap water, sterilized in 0.5 % NaOCl for 1 min and dried with sterile filter paper. Once disinfected, the tissue was cut into 3-4 mm pieces and placed onto Petri dishes containing Alcohol Water Agar (1.0 litre distilled water, 8.0 g agar, 5.0 ml alcohol), PDA, and Nash and Snyder Medium (15.0 g peptone, 1.0 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 20.0 g agar, 1.0 g PCNB, 1.0 liter distilled water). The plates were incubated at 24°C (7,10).

Determination of inoculum density of V.dahliae. Soil samples (200g) were taken from 20 cm of top soil in each field. Three, five and seven soil samples were taken from 1.0, 1.0-5.0 and 5.0-10.0 ha fields, respectively, mixed, air dried for 1-2 weeks at 25°C and passed through a 2 mm screen. Inoculum density of *V.dahliae* in the samples was detected by a soil dilution technique (Mol *et al.*, 1996). In this method, 25 gm of soil from each samples is mixed in 75 ml

sterile distilled water. Soil suspensions are shaken at 270 rpm for 1 hour and 1.0 ml soil suspension of 10_3 dilution is distributed on MSEA -Modify Soil Extract Agar (Harris *et al.*, 1993). Plates are incubated for 14 days at 23°C in the dark and then soil residues are washed with water from agar surface in plates. Plates are dried on the bench and incubated for 2-3 weeks. After that, V. dahliae colonies on MSEA are enumerated under a steoroscopic microscope and the microsclerotia per gram of soil is calculated.

Pathogenicity. Isolates of *V.dahliae* and *Fusarium* from cotton plants were used for the pathogenicity tests. Eggplant cultivar Pala for *V. dahliae* isolates and cotton cultivar Cukurova 1518 for *Fusarium* isolates were chosen as test plants. One-month old seedlings about 5-6cm height were inoculated by dipping into inocula.

The isolates of *V.dahliae* and *Fusarium* were grown on PDA slants for 7 days at 24°C in the dark as inoculum. Conidial suspensions from the slants were adjusted to 10_6 conidia/ml with a hemacytometer. Seedlings were removed and roots were washed under tap water and dipped in conidial suspension for 1-2 minute. Inoculated plants were transplanted into sterile soil pots. Inoculated seedlings were grown at 16-h light and 8-h dark period, at, 24-25°C for 8-10 weeks. Finally, all plants were cut off at soil level and evaluated for vascular discoloration and foliar symptoms.

Differentiation of strains of V. dahliae. Giza 75 (resistant) of *G. barbadense* and Acala 1517/V (moderately resistant), Deltapine 15/21 (susceptible) of *G. hirsutum* were used as differential cultivars. Five plants of each cultivar were inoculated with each isolate when they had 4-5 true leaves. Plants were inoculated using the stem puncture technique. Inoculums of *V. dahliae* isolates were grown in PDA slants for 7-10 days and the number of conidia per ml of suspension was adjusted to $3x10_6$ with a haemocytometer. Conidial suspensions of each strain were injected separately with a sterile 1-ml hypodermic syringe between the cotyledons and first true leaves. Diseased plants and disease severity were recorded at 10, 15, 20 and 50 days after inoculation were recorded. Disease severity on a 0-4 scale -0, no visible symptoms; 4, dead or nearly dead plant (Wilhelm, 1974).

V.dahliae strain differentiations were also determined at optimum temperature for growth of the pathogen. *V. dahliae* isolates from infested cotton-growing area, were transferred to PDA plates amended 200mg/l streptomycine sulfate. Plates were incubated at 24 and 27°C. Growth rates wee recorded as colony diameters were measured at 3- 4 day intervals for 14 days (Schnathorst *et al.*, 1975.

Results and Discussion

Disease surveys. *Verticillium* wilt was found in 11 fields in 1994 and 12 fields in 1995 (Table 1). During surveys, *Verticillium* wilt showed epinasty, chlorosis and necrosis on the leaf, early maturing of plants, reduced plant growth, defoliation, stunting and light to dark brown vascular discoloration in the mean stem, root, and branches of diseased plants. *Verticillium* wilt disease was observed in only four counties (Karatas, Ceyhan, Yuregir and Imamoglu) in 1994 and in five (Ceyhan, Yuregir, Imamoglu, Osmaniye and Kozan) in 1995. Incidence of *Verticillium* wilt per field was highest in Yrg 3 field in Yuregir in 1994-1995 at 61.4%. The mean cotton wilt disease prevalence in Adana was 14.3% in 1994 and 30.1 % in 1995 and the incidence was 13.9 % in 1994 and 13.0 % in 1995 (Table 2).

Cotton has been cultivated for 10-15 years in successive seasons without rotation in the wilted areas. Cukurova 1518, the main cultivar susceptible to *V. dahliae* wilt, is extensively grown in Adana (Dolar, 1984). The soil texture of surveyed fields was clay in Ceyhan, Yuregir, Imamoglu and Osmaniye, clay-loam in Kozan and silt-clay-loamy in Karatas. For these reasons, crop rotation (cotton- wheat or barley) is suggested (Evans *et al.*, 1967). Using excessive nitrogen specifically in the form of nitrate, often at 50kg/da, increases the wilt incidence and prevalence. It was determined that *Verticillium* wilt severity is correlated positively with the availability of nitrogen (El-Zik, 1985; Ranney, 1973).

The colour of isolated colonies from diseased cotton plant tissues were first white, later converting to black with microsclerotial production. Colony diameter reached up to 19 to 26 mm after 7 days at 24°C. Conidiophores were erect, septate and branched and hyaline. Conidia were unicellular, ellipsoid and hyaline about 3-5x1.2-3.1 µm in diameter. The properties of conidia, conidiophores and presence of microsclerotia suggested that the causal agent of wilted cotton plants is *V. dahliae* (Domsch *et al.*, 1980; Melouk, 1992). Black microsclerotia are used as a criterion for differentiation of *V. dahliae* from *V. albo-atrum* (Isaac, 1967). Similer results were previously reported in Adana, Karatas and Osmaniye (Esentepe, 1979). Twenty three *V. dahliae* isolates were recovered from cotton plants in Adana in 1994-1995 in addition to isolates of *F. oxysporum*, *F. solani*, *F. semitectum* and *F. moniliforme*.

Inoculum density in soil. Inoculum assessment of *V. dahliae* in soil samples on MSEA from each county showed generally more than 100 microsclerotia/g soil. The highest inoculum density was found in Kozan with 195.4 microsclerotia/g soil while the lowest was in Osmaniye with 48.8 microsclerotia/g soil and the mean infested soils in cotton growing areas of the Adana region was 129.5 microsclerotia/g soil. The microsclerotia in the soil were found about at the same level (200 microsclerotia/g soil) in cotton fields (Pullman and De Vay, 1982). At least 100 microsclerotia are needed to cause wilting symptoms on cotton plants. Similar results were reported by

Schnathorst (1981). Increase in inoculum density from 5 to 60 microsclerotia/g soil resulted an increase in the percentage of infected plants from15 to 95 %, respectively (El-Zik, 1985).

Cotton cultivation in the same fields for a long period will increase inoculum density of *V. dahliae* in soil. The mean inoculum increase in infested fields was 13-15 propagules per g of soil for each year under cotton. If cotton growing is repeated fir 2-3 successive seasons in field initially showing 5-10% disease incidence, wilt can increase to 80-90% (Pullman and De Vay, 1982).

Pathogenicity. Three isolates of *F. oxysporum* and other *Fusarium* species were non-pathogenic to cotton plants. In the pathogencity test with 23 different isolates of *V. dahliae,* epinasty and interveinal chlorosis type of symptoms were observed on inoculated plants. Infected leaf areas turned necrotic and defoliation occurred severely in some inoculated plants. All of the *V. dahliae* isolates were pathogenic to the indicator eggplant. *V. dahliae* reisolated from all previously inoculated plants.

Differentiation of Strains. Differentiation of the *V. dahliae* strains was done, based on symptom expression in the inoculated differential cotton cultivars at optimum temperature for growth (Table 3). Six of the isolates were T-1 and the other six were SS-4. Isolates T-1 grew optimally at 27°C and caused defoliation and epinasty on differential cultivars. T-1 isolates exhibited wilt symptoms in all cultivars within 15-20 days and plants with epinasty defoliated 10 days after inoculation and some plants died completely (Table 3).

The other 6 isolates of SS-4 strain grew optimally at 24°C. Within 15 days after inoculation, the lower leaves of cultivars Deltapine and Acala showed chlorosis followed by necrosis after 20 days. Dead leaves remained attached to the plants. Several wilt symptoms were observed in Deltapine (highly wilt susceptible), whereas Acala exhibited a high degree of tolerance to SS-4. Although Giza-75 appeared to be unaffected externally, internal vascular discoloration appeared in a few plants. Twelve of the 23 isolates were SS-4 and the other 11 were T-1 in the experiments.

These results support those of Schnathorst and Mathre (1966) who used the same differential cultivars to determine that *G. hirsutum* cultivars are susceptible and that *G. barbadense* cultivars are low tolerant to T-1. The same cotton cultivars inoculated with SS-4 were moderately susceptible. This indicated that the complete series of cultivars should be used to differentiate two types of the isolates. Acala and Deltapine cultivars have often been used in differentiation of strains (Schnathorst and Evans, 1971; Schnathorst and Foggle, 1973; Schnathorst *et al.*, 1975; Schnathorst and Sibbet, 1971).

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Table 1. Results of cotton wilt survey conducted in 1994-1995 in Adana Province.

County	Surveyed fields		Diseased fields and area (da)		Area of surveyed fields (da)	
	1994	1995	1994	1995	1994	1995
Karatas	18	18	2 (155)	0	800	650
Ceyhan	14	14	3 (70)	2 (65)	300	275
Yuregir	12	12	4 (173)	3 (160)	430	300
Karaisali	9	9	0	0	275	250
Yumurtahk	8	8	0	0	330	240
Imamoglu	10	10	2 (74)	3(135)	139	135
Kadirli	3	3	0	0	155	120
Osmaniye	3	3	0	2 (55)	95	105
Kozan	2	2	0	2 (110)	85	70
Ceyhan	2	2	0	0	15	35
Duziri	1	1	0	0	10	15
Total	82	82	11 (472)	12(525)	2634	2225

Table 2. Disease incidence and prevelance in some counties of Adana in 1994-1995.

County	Incidence of Disease %		Prevelance of Disease %	
	1994	1995	1994	1995
Yuregir	30.0	28.4	33.3	25.0
Ceyhan	14.1	5.7	21.4	14.2
Imamoglu	9.6	23.6	20.0	30.0
Karatas	29.7	0.0	11.1	0.0
Osmaniye	0.0	4.5	0.0	66.6
Kozan	0.0	15.5	0.0	50.0
Mean	13.9	12.9	14.3	30.9
Weighted Mean	22.8	9.9	-	-

Table 3. Differentiation of strains of 12 *V. dahliae* isolates from cotton in Adana.

Isolate No	Response of differential cotton cultivars			Optimum Temp.	Strains
	Acala 1517/V	Deltapine 15/21	Giza 75		
Im3	Lethal,defoliation	Lethal,defoliation	Lethal,defoliation	27₀C	T-1
Im4	Lethal,defoliation	Lethal,defoliation	Lethal,defoliation	27 ,,	T-1
Ko3	Lethal,defoliation	Lethal,defoliation	Lethal,defoliation	27 ,,	T-1
Yür1	mild	Lethal, no defol.	No lethal, no defol.	24 ,,	SS-4
Yür2	mild	Lethal, no defol.	No lethal, no defol.	24 ,,	SS-4
Yür6	Lethal,defoliation	Lethal,defoliation	Lethal,defoliation	27 ,,	T-1
Yür7	mild	Lethal, no defol.	No lethal, no defol.	24 ,,	SS-4
Ch6	Lethal,defoliation	Lethal,defoliation	Lethal,defoliation	27 ,,	T-1
Ch9	Lethal,defoliation	Lethal,defoliation	Lethal,defoliation	27 ,,	T-1
Kar2	mild	Lethal, no defol.	No lethal, no defol.	24 ,,	SS-4
Im5	mild	Lethal, no defol.	No lethal, no defol.	24 ,,	SS-4
Os1	mild	Lethal, no defol.	No lethal, no defol.	24 ,,	SS-4