



Development of *Verticillium dahliae* in Cotton Plants Grown in Cukurova and Reaction of Some Cultivars to Wilt

S. Kurt and M. Bicici

University of Cukurova, Department of Plant Protection, Adana-Turkey

ABSTRACT

Seven cotton cultivars were inoculated with *Verticillium dahliae* Kleb. using a spore suspension of 2.5×10^6 conidia/ml by the stem puncture technique. Infection development of symptoms on plants inoculated with T-1 and SS-4 strains were investigated until the 25th day after inoculation. Strain T-1 reached 15 cm above the inoculation point in 7 days in all cultivars tested except Cukurova 1518, while strain SS-4 moved the same distance in all cultivars except Sayar 314 in the same period. On 21st day after inoculation, strain T-1 was isolated 30 cm away from inoculation point in all cultivars except Sayar 314 and DPL50 but strain SS-4 was isolated from 17 cm away in all cultivars except Sayar 314. The results showed that strain T-1 progressed faster than isolate SS-4 in the first 21 days. However, by the 25th day, infection development for both isolates was about the same with 30 cm movement in all cultivars. Disease severity was recorded as foliar symptoms and vascular discoloration in both greenhouse and field conditions. Cultivars Deltapine 15/21, Maras 92, Sayar 314 and Ersan 92 were highly susceptible to T-1 in the greenhouse while cultivar Deltapine 15/21 was highly susceptible to SS-4. Acala cultivars were moderately resistant to SS-4. Cukurova 1518, a standard cultivar, showed little resistance to SS-4. The *G. barbadense* cultivars Giza 45, Giza 75 and Askabat were resistant to T-1 in the field. Amongst *G. hirsutum* cultivars, Acala was moderately resistant, while all other cultivars were susceptible to T-1. All cultivars except DPL15/21, Cukurova 1518 and Sayar 314 were highly or moderately resistant to SS-4.

Introduction

Cotton is the most important export crop and one of the basic raw materials of textile and oil industries in Turkey. The area sown to cotton has decreased recently in Cukurova region and now stands at around 250,000 ha. (Anon, 1997). Diseases and pests are the most important limiting factors. Cotton wilt caused by *Verticillium dahliae* is recognized as one of the most serious diseases. Control of the disease is best achieved by tolerant cultivars and cultural measures, due to difficulty of chemical control. Wilt disease has received much attention from researchers throughout the world, but very little has been reported on its pathological anatomy in cotton (Garber and Houston, 1966). Infection of the cotton plant with *V. dahliae* may result either from direct penetration of young uninjured roots or through root and stem wounds. The fungus penetrates the epidermis and grows intercellularly through the cortex until it reaches the xylem (Garas *et al.*, 1986). It can be isolated from the cotyledons of cotton plants before it could be isolated from the upper taproot or the hypocotyl (Garber and Houston, 1967). In this study, the development of infection of *V. dahliae* in different cotton cultivars and the reactions of the cultivars to the pathogen were studied in greenhouse and field conditions.

Material and Methods

Growth of *V. dahliae* in cotton plants. Seedlings of cultivars Maras 92, Sayar 314, Ersan 92, Cukurova

1518, Deltapine 50, Deltapine 20 and Carolina Queen were inoculated when they were about 8 weeks old (4-5 true leaves). The Kar-2 isolate of SS-4, a mild, nondefoliating strain and Ch-5 of T-1, a defoliating strain of *V. dahliae*, isolated from infected cotton plants, were used. Cultures were incubated on PDA slants at 24-25°C for 7-10 days. Conidial suspensions were prepared from 4-5 day old cultures and adjusted to 2.5×10^6 conidia/ml.

The stem-puncture technique was used to introduce the conidia directly into the xylem tissues. Three stem-puncture inoculations were made in the hypocotyls of cotton plants with a hypodermic needle containing 0.2 ml of the concentration of 2.5×10^6 conidia/ml. Control plants were treated only with sterile water. Inoculated plants were grown at ambient conditions. Samples were taken daily in the first 7 days from the stem and the leaf petiole at the first, second and third internodes and cultured. The stem and petiole samples were sectioned (1-2 mm) and surface sterilized in 2 % NaOCl for 2 min., rinsed in sterile distilled water and dried with sterile paper. Tissues were plated onto Plum Lactose Agar (PLA) and incubated at 24°C for 7 days. Plates were then examined for characteristic growth, sporulation and microsclerotial formation (Garas and Waiss, 1986; Garas *et al.*, 1986). In other experiment concerning infection period for regional cultivars, symptoms which occurred 7, 14, 18, 21 and 25 days after inoculation with both strains were evaluated.

Reaction of the cotton cultivars to Verticillium wilt.

Two isolates belong to mild SS-4 and defoliating T-1 strain of *V. dahliae* obtained from diseased cotton plants were selected. Cultures were grown on *Verticillium* minimal medium at 25°C for 3-4 weeks (Melouk, 1992). Experiments were conducted in both greenhouse and field conditions. Cultivars tested were Giza 45, Giza 75 and Aşkabat 71 of *G. barbadense* and Deltapine 15/21, Deltapine 20, Deltapine 50, Acala 1086, Acala 1517/V, Acala 1517/D, Çukurova 1518, Carolina Queen, Sayar 314, Maraş 92 and Erşan 92 of *G. hirsutum*.

Pot tests. Seeds were sown in 15cm- diameter pots containing soil and manure (1/1; v/v). With one seedling in each pot (using Kar-2 and Ch-5 isolates), the experiment was established in a randomized block design with 5 replicates and repeated 4 times. Each seedling was inoculated as described when they were about 30-40 cm tall. Inoculated plants were grown in ambient conditions and wilt symptoms were observed (Fasihiani, 1995; Friebertshauer and de Vay, 1982; Hamdallah-Zadeh, 1995). From 15 days after inoculation, the plants were scored for wilt symptoms at intervals of 10, 20, 30 days from flowering stage to harvesting. The wilt value was recorded according to the degree of chlorosis or wilting symptoms using 0-4 scale (0, indicated the absence of visible symptoms; 4 a dead or nearly dead plant). The intermediate values of 2 and 3 indicated degrees of leaf yellowing, interveinal necrosis, defoliation and terminal dieback. The score of 1 indicated slight or questionable symptoms such as incipient wilting or slight yellowing of leaves.

At harvest, the stem of each plant was cut near the ground and rated on a scale of 0-4 for intensity and pattern of vascular discoloration along 40 cm height of stem. A score of 0 indicated the absence of discoloration; 1, very slight streaking in the wood nearest the pith; 2, slight streaking distributed sporadically throughout the wood; 3, distinct dark discoloration throughout the wood and 4, intense uniform discoloration and wood deterioration *Verticillium* (Wilhelm *et al.*, 1974).

Disease severity on each pot was evaluated by using index formula. Plant wilt score or average wilt score of a cotton cultivar was based on the scores for symptoms and for vascular discoloration, and success of isolation of *Verticillium* from petioles. If *Verticillium* was isolated from petioles during the growing season or from discolored wood at the end of the season, the value of 1 was added to the symptom score. Wilt scores of 0-3.5 indicated resistance; 4-5, susceptibility. The score of 3 was considered to represent the minimal resistance necessary to control the severe defoliating strain of *Verticillium* (Wilhelm *et al.*, 1974).

Field tests. Cultivars used in the pot tests were screened to both the SS-4 and T-1 in field conditions. Experiment was designed as completely randomized

blocks with four replications. Plants were inoculated using the same strains, spore suspension and inoculation technique when they were at the 5 to 6 true leaf stage. From 15 days after inoculation, all plants were scored on a scale of 0-4 (Wilhelm *et al.*, 1974) at intervals of 20-30 days until harvest. For foliar symptoms, the mean disease index of three evaluation was calculated. At harvest, the main stem of each plant was cut near the ground at a height of about 40 cm and a disease index calculated, based on the scores for vascular discoloration. If *Verticillium* was isolated from petioles, the value of 1 was added to the symptom score.

Results and Discussion

Infection development of V. dahliae in the cotton plants

Development of T-1 and SS-4 strains in cotton tissues one week after inoculation. Isolate Ch-5 (T-1) reached to 10 cm 5 days after of inoculation. *V. dahliae* was isolated at 15cm above the inoculation point in all cultivars except C 1518. Three days after inoculation with Kar-2 isolate (SS-4), infection reached 5 cm in all cultivars. *V. dahliae* was isolated at 10cm by the fifth day in all cultivars and at 15cm in the seventh day in all cultivars except Sayar 314. The results showed that the rate of spread of T-1 and SS-4 in the plant were similar during the first week after inoculation. In a similar study, the Acala and DPL cultivars showed no difference in relation to growth of the fungus at the entry point of the pathogen into xylem. The number of colonized vessels was correlated with mycelial growth and the number of the infection sites (Garber and Houston, 1966). More vessels were colonized and more free floating conidia were present in the susceptible (DPL15) than in the resistant cultivar (Acala) (Garber and Houston, 1967).

Infection development of the T-1 and SS-4 at different five days until 25th day of inoculation. The development of the Ch-6 isolate (T-1) of *V. dahliae* upward from the inoculation point was determined by isolations from stem and petiole samples taken from 10, 20 and 30 cm (14 days after inoculation). The fungus reached 15cm from the inoculation point in all cultivars. In 21 days after inoculation, all cultivars except Sayar 314 and DPL50 were colonized to 30 cm above the inoculation point. The fungus reached 30cm above the inoculation point in all cultivars 25 days after inoculation.

SS-4 strain (Kar-2) was detected at 5 cm above the inoculation point in all cultivars seven days after inoculation. It was isolated from 10 cm 14 days of inoculation. The SS-4 strain reached 17 cm in all cultivars except Sayar 314 by 21 days after inoculation and 30 cm in all cultivars by 25 days of inoculation. The progress of infection of the both strains was similar and the vascular tissues were colonized by both strains up to 30 cm by 25 days after inoculations. These

results agree with previous work (Garas and Waiss, 1986) which indicated that the pathogen (T-1 and SS-4) was isolated less frequently from the resistant Seabrook Sea Island than from the tolerant or susceptible cotton cultivars.

Reaction of the cotton cultivars to *Verticillium* wilt

Pot tests. Reaction of cultivars to both T-1 and SS-4 strains was tested by inoculation of cotton seedlings. The T-1 strain caused chlorosis about one week after inoculation, followed by necrosis of infected area and severe defoliation. Based on foliar symptoms, the cultivars Maras 92, DPL 15/21, Sayar 314, Ersan 92 and C.1518 had the highest index value with 3.75, 3.63, 3.38, 3.38 and 3.37, respectively. Cotton resistant cultivars such as Giza 45, Giza 75 and Askabat 71 inoculated with SS-4 did not show any conspicuous foliar symptom of *Verticillium* wilt. On the other hand, on the susceptible cultivars such as DPL 15/21, the dead leaves remained attached to the plant and showed chlorosis and necrosis. The cultivars DPL 15/21, C.1518, C. Queen and DPL20 had the highest index value for vascular discoloration with 2.88, 2.38, 2.38 and 2.25, respectively.

In response to inoculation with the T-1 strain, Acala 1517/D and Acala 1517/V showed slight resistance, Giza 45, Giza 75, DPL50 and Aşkabat 71 were resistant and others were susceptible. With the SS-4 strain, cultivars Giza 45, Giza 75, DPL50 and Aşkabat 71 were highly resistant; Acala 1086, Acala 1517/D and Acala 1517/V showed intermediate resistance; DPL20, Sayar 314, Ersan 92, C.1518, Maras 92 and Carolina Queen slight resistant and DPL 15/21 was highly susceptible. All data indicated that *G. barbadense* cultivars were generally more resistant than *G. hirsutum* cultivars to *Verticillium* wilt. This result supports findings of Wiles (1953) working on the reaction of the cotton plants to *Verticillium* wilt. However, it was found that *G. hirsutum* cultivars showed different reactions to the wilt pathogen when using different strains of the pathogen in the greenhouse (Dolar, 1984; Karcilioğlu *et al.*, 1982; Karcilioğlu *et al.*, 1992). These results agree with the current findings.

Field tests. Cultivars Giza 45, Giza 75 and Aşkabat 71 had resistance score 1-2 to T-1 while cultivars Acala 1517/D, Acala 1517/V and DPL50 had resistance scores 2-3 to T-1. All the other cultivars were susceptible. In the reaction studies of the same cultivars to SS-4, the cultivars Giza 45, Giza 75 and Askabat 71 with resistance scores 0.5-1.5 were highly resistant, cultivars Acala 1086, Acala 1517/D, Acala 1517/V, DPL 50, Maras 92, C. Queen, DPL20 and Ersan 92 had resistance scores of 2.0-2.5, while cultivars DPL 15/21, C.1518 and Sayar 314 had resistance scores of 2.5-3.5 (Table 1).

Pot and field tests showed that *G. barbadense* cultivars were resistant to the strains T-1 and SS-4. Cukurova 1518, a standard *G. hirsutum* cultivar, was susceptible

to T-1 and slightly resistant to SS-4. The different reactions of some cotton cultivars to the T-1 and SS-4 both in the pot and in field tests could have arisen from environmental factors. In the field tests in particular, soil and air temperatures above 30°C may effected the test results.

References

- Anonymous, (1997): Pamuk ekim alanları ve verim. Tarım ve Köyişleri Bakanlığı İl Müdürlüğü İstatistik Şubesi, ADANA.
- Dolar, M.S. (1984): Akdeniz bölgesi pamuklarında görülen Solgunluk hastalığı (*Verticillium dahliae* Kleb.)' na karşı bazı pamuk çeşitlerinin duyarlılıklarının saptanması üzerine çalışmalar. Bitki Koruma Bülteni, 24:148-158.
- Fasihiani, A. (1995): Reaction of some cotton cultivars to *Verticillium dahliae*. In: Proceeding of the 12th Iranian Plant Protection Congress, 2-7 September. Karadj-Iran.
- Friebertshausen, G.E. and J.E. DeVay. (1982): Differential effects of the defoliating and nondefoliating pathotypes of *Verticillium dahliae* upon the growth and development of *Gossypium hirsutum*, *Phytopathology*, 72:872-877.
- Garas, N.A. and A.C. Waiss Jr. (1986): Differential accumulation and distribution of antifungal sesquiterpenoids in cotton stems inoculated with *Verticillium dahliae*. *Phytopathology*, 76:1011-1017.
- Garas, N.A., S. Wilhelm and J.E. Sagen. (1986): Relationship of cultivar resistance to distribution of *Verticillium dahliae* in inoculated cotton plants and to growth of single conidia on excised stem segments. *Phytopathology*, 76:1005-1010.
- Garber, R.H. and H.R. Houston. (1966): Penetration and development of *Verticillium albo-atrum* in the cotton plant. *Phytopathology*, 56:1121-1126.
- Garber, R.H. and H.R. Houston. (1967): Nature of *Verticillium* wilt resistance in cotton. *Phytopathology*, 57:885-888.
- Hamdallah-Zadeh, A. (1995): Evaluation of the reaction of super crosses and new cultivars of cotton to *Verticillium* wilt. In: Proceeding of the 12th Iranian Plant Protection Congress. 2-7 September. Karadj-Iran.
- Karcilioğlu, A., M. Esentepe and E. Sezgin. (1982): Investigations on the determination of susceptibility of some cotton varieties against cotton wilt disease caused by *Verticillium dahliae* Kleb. *J. Turkish Phytopathology*, 11:55-59.
- Karcilioğlu, A., E. Onan, H.B. Kabadayı and I. Naza. (1992): Behaviour of some cotton varieties to

cotton wilt diseases caused by *Verticillium dahliae*
Kleb. J. Turkish Phytopathology. 21:49-53.

Melouk, H.A. (1992): *Verticillium*. In: Methods for Research on Soilborne Phytopathogenic Fungi. I.I. Singleton, J.D. Mihail and C.M. Rush (Ed). APS Press, St Paul, MN. USA. 265 pp.

Wiles, A.B. (1953): Reactions of cotton varieties to *Verticillium* wilt. Phytopathology Annual Meeting Abstract, 43:489.

Wilhelm, S., J.E. Sagen and H. Tietz. (1974): Resistance to *Verticillium* wilt in cotton: sources, techniques of identification inheritance trends and resistance potential of multiline cultivars. Phytopathology, 64:924-931.

Table 1. The mean index value of *V. dahliae* strains on different cultivars in field tests.

Cultivars	Foliar symptoms		Vascular discoloration	
	T-1	SS-4	T-1	SS-4
C.1518	2.80 ab	2.30 a	3.13 a	2.45 ab
Maras 92	2.60 abc	2.05 ab	2.55 abc	1.38 cdef
Sayar 314	2.90 a	2.23 ab	3.05 a	2.65 a
Ersan 92	2.80 ab	2.03 ab	2.95 a	1.73 cde
C. Queen	2.30 cd	1.79 bcd	2.88 ab	1.73 cde
DPL 15/21	2.60 bc	1.80 bcd	2.58 abc	1.90 bcd
DPL 20	2.40 cd	1.78 ab	2.80 abc	1.88 bcd
DPL 50	2.60 abc	2.01 ab	2.55 abc	1.65 cde
A.1086	2.80 ab	2.18 ab	2.63 abc	2.05 abc
A. 1517/D	2.60 abc	1.93 abc	2.05 bcd	1.33 def
A. 1517/V	2.40 cd	1.93 abc	1.98 cd	1.3 def
Askabat 71	2.20 de	1.52 cd	1.40 d	0.80 f
Giza 75	2.10 de	1.44 d	1.45 d	0.73 f
Giza 45	2.20 e	1.53 cd	1.30 d	0.90 f

*Means followed by the different letters are statistically different (P=0.05) using Duncan's multiple range test