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Evaluation of Verticillium Wilt Tolerance in Upland Cotton Relative to Lint Yield Reduction[1]

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ABSTRACT

Field experiments were conducted to compare methods of evaluating the tolerance of upland cotton (*Gossypium hirsutum* L.) to *Verticillium dahliae* Kleb. by visual symptoms. Good differentiation between susceptible and tolerant cultivars was obtained by observing foliar symptoms after the middle of the flowering period.

Correlation studies indicated that foliar symptoms explained 31 to 45% of the variation in lint yield among cotton breeding lines and cultivars in a wilt-infested field, vascular discoloration at the base of the stem explained 25 to 29%, and vascular discoloration at a height of 40 cm explained 6% or less of that variation. Similar results were obtained when the dependent variable was the difference between lint yield in the infested field and mean lint yield of the same lines grown in two wilt-free locations. Effective screening for wilt tolerance in Israel appears possible by observing foliar symptoms during the second half of the flowering period, provided the field is relatively uniformly infested by the fungus and suitable temperatures prevail.

Additional index words: Gossypium hirsutum L., *Verticillium dahliae* Kleb.

VERTICILLIUM wilt of cotton (*Gossypium hirsutum* L.), caused by *Verticillium dahliae* Kleb. (also referred to as the microsclerotial form of *V. albo-atrum* Reinke & Berth.), induces severe losses of lint yield in many countries. No absolute resistance to this disease is known in upland cotton, but some cultivars are more tolerant to the disease than are others. Bell (4) reviewed the extensive literature on the nature of Verticillium wilt tolerance in cotton and reported that resistance increases with the age of the plant after the four-to-six true-leaf stage, and maximum resistance usually develops 2 to 4 weeks after initial flowering. A decrease in resistance occurs later when the fruit load develops and the cambium becomes dormant.

Garber and Houston (6, 7) found that the level of wilt tolerance does not affect he number of vessel elements invaded by the fungus at primary invasion sites, but more vessels are colonized at higher points on the stalk and more free-floating conidia are found in susceptible cultivars than in tolerant ones. Bell (4) suggested that the primary difference between susceptible and tolerant *G. hirsutum* cultivars may be the differential sensitivity of the xylem parenchyma cell membranes to fungal toxins and the resulting differential leakage of nutrients into xylem vessels. As a consequence, the fungus colonizes tolerant cultivars more slowly, and this would allow resistant responses to be more effective in containing secondary colonization.

Barrow (2) reviewed the research on the genetics of Verticillium tolerance in cotton. It seems fairly well established that in 'Acala' cultivars a dominant gene controls tolerance to a mild Verticillium strain (SS4) under field and laboratory conditions. However, several other sources of tolerance are known, and different types of gene action for tolerance exist. The strain of the pathogen, amount of inoculum, temperature, and other environmental factors may modify the expression of symptoms on the cotton plant and complicate the genetical interpretation.

Cotton breeders are faced with the problem of efficiently selecting the most tolerant lines among their breeding materials. Verticillium tolerance is associated in many cases with strong vegetative growth, late maturity, and a small fruit load. However, these are unfavorable agronomic characteristics and should be avoided because of their negative effects on lint yield. Therefore, any method of evaluation based on external observable disease symptoms should also indicate the expected effect of disease tolerance on lint yield. Evaluation of disease symptoms is usually based either on foliar symptoms or vascular discoloration. The time of foliar rating is an important factor in determining differential tolerance. Several workers (3, 8, 9) have previously correlated cotton yields with various methods of Verticillium rating.

The purpose of the present study is to determine which method of screening cotton for wilt tolerance is most highly associated with lint yield performance in infested fields, and consequently to recommend the use of that method in future breeding efforts.

MATERIALS AND METHODS

Preliminary field observations were taken in 1971 at Dorot (in the northern Negev region of Israel) in a field infested with Verticillium and in which the previous crop of potatoes (*Solanum tuberosum* L.) had shown severe disease symptoms. Susceptible and tolerant cultivars of upland cotton were randomly planted among cotton breeding plots. The susceptible cultivars were 'Deltapine Smoothleaf' (DPSL) and 'Carolina Queen' (CQ) and the tolerant cultivars were 'Acala-4-42' (4-42), 'Acala SJ-1' (SJ-1) and 'Deltapine 5540' (DP 5540). SJ-1 was planted in four plots, and all the others in three plots apiece. Each plot consisted of approximately 25 plants in a single row 3 m long with rows spaced 97 cm apart. Cotton was planted on 3 May and subsequently irrigated. Three additional irrigations were applied in July and August. The number of plants showing definite Verticillium symptoms on their leaves was recorded for each plot on the following dates: 25 June; 9, 15, and 25 July; 4, 11, and 25 August.

Two field experiments were conducted in 1972 at Dorot. *Exp. A* consisted of 72 entries including F_5 and F_6 lines derived from the crosses 4-42 × DPSL and 1517C × DPSL, as well as several check cultivars. An 8 × 9 rectangular lattice design with three replications was used. *Exp. B* included 81 entries of check cultivars and F_4 lines derived from nine different crosses between 4-42, 1517C, SJ-1, DPSL, CQ, and 'Del-Cerro'. A 9 × 9 simple lattice design replicated six times was used. Both experiments were planted near the middle of April; each plot consisted of one row 3 m long with 8 to 10 plants/m of row, and rows were

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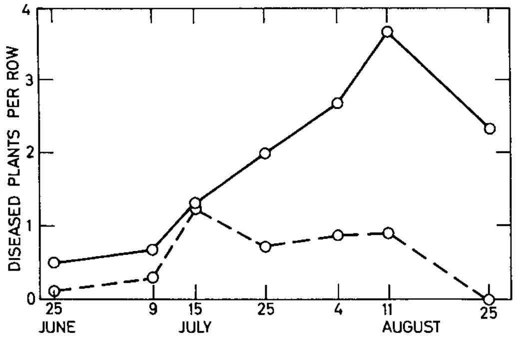

Fig. 1. Mean number of plants/row exhibiting foliar symptoms of Verticillium wilt on several observation dates at Dorot in 1971. [— — — — Tolerant cultivars (4-42, SJ-1, and DP-5540); ——— Susceptible cultivars (DPSL, and CQ)].

97 cm apart. The field was irrigated four times from the middle of June to the beginning of August. Foliar symptoms of Verticillium appeared on almost all plants in these experiments. Symptoms were scored on 11 and 28 July; i.e., in the middle and near the end of the flowering period. Plots were scored using the following scale: 0 = no visual symptoms; 1 = slight foliar symptoms; 2 = moderate foliar symptoms; and 3 = very severe foliar symptoms. In some cases intermediate grades (i.e., 0.5, 1.5, 2.5) were assigned.

Plots were harvested by hand-picking, and lint yield was determined after ginning. After harvest, 10 random plants were taken from each plot and rated for vascular discoloration by a method similar to that described by Wilhelm et al. (10). The stalks were cut near the ground, and at a height approximately 40 cm above ground level. The sections were examined for intensity and pattern of vascular discoloration using the following grades: 0 = no discoloration; 1 = very slight browning in some spots, 2 = slight to medium browning in streaks; 3 = dark browning covering most of the wood; and 4 = intense uniform discoloration. A mean score was then calculated for each plot.

In each experiment, linear correlation and regression coefficients were calculated between lint yield and the several methods used for evaluating wilt symptoms, based on the mean values of the entries. The same breeding lines were grown the same year in identically designed experiments at two wilt-free locations (Givat-Brenner and Beisan). The difference between the mean yield of a line at these two locations and its yield at Dorot was taken as an indicator of relative decrease in lint yield due to Verticillium susceptibility. Linear correlation and regression coefficients were also calculated between this difference and the several methods used for evaluating wilt symptoms.

RESULTS

Results of the preliminary field observations at Dorot in 1971 are given in Fig. 1. Differences between cultivars within the susceptible group or the tolerant group were very small. Therefore, mean results for each group of cultivars are presented. There was no significant difference between the cultivar groups until the middle of July. At the end of July and during August, the number of plants showing wilt symptoms increased consistently in the susceptible cultivars but not in the tolerant ones. Even though only about 16% of the plants of susceptible cultivars exhibited foliar symptoms, presumably because Verticillium infestation was not uniform in this field, nevertheless, a clear difference between the susceptible and the tolerant cultivars was found during this period. This period coincided with the setting and development of most bolls. Calculated t values for differences between cultivar groups (4.64, 3.53, 5.77, and 6.30, for 25 July, 4, 11, and 25 August, respectively) were highly significant. The number of apparently infected plants decreased after the end of August. This was probably caused by the shedding of infected leaves whereas subsequent leaves did not show any observable disease symptoms.

Results of the 1972 experiments are presented in Tables 1, 2, and 3. Foliar symptoms were significantly and negatively correlated with lint yield (Table 1). The squared correlation coefficient (r^2) indicated that these symptoms explained 31 to 45% of the variation in lint yield found between the breeding lines in these experiments. Other factors contributing to this variation were genetical differences not associated with wilt tolerance, as well as random environmental effects

Table 1. Linear correlation and regression coefficients between methods of grading for Verticillium wilt symptoms and lint yield of cotton breeding lines and check cultivars.

Dependent variable (kg/ha)	Independent variable	Coefficient of correlation, r		Coefficient of linear regression and its standard error	
		Exp. A	Exp. B	Exp. A	Exp. B
Lint yield	Foliar rating				
	11 July	-0.56**	-0.67**	-357 ± 63	-403 ± 51
	28 July	-0.56**	-0.61**	-375 ± 66	-342 ± 50
	Vascular rating				
	40 cm	-0.08	-0.25*	- 50 ± 75	-175 ± 76
	base	-0.50**	-0.54**	-362 ± 74	-336 ± 59
Reduction of lint yield†	Foliar rating				
	11 July	0.57**	0.67**	337 ± 58	390 ± 49
	28 July	0.61**	0.62**	375 ± 58	332 ± 49
	Vascular rating				
	40 cm	0.14	0.30**	81 ± 69	203 ± 71
	base	0.53**	0.57**	355 ± 67	338 ± 55

*,** Significant at the 0.05 and 0.01 probability levels, respectively.
† Difference between the mean lint yield of each line at two wilt-free locations and its lint yield at Dorot.

Table 2. Minimum and maximum values, means, and standard deviations of the variables measured.

Variable	Exp. A				Exp. B			
	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
Foliar rating								
11 July	1.0	2.7	1.7	0.4	0.8	2.7	1.7	0.5
28 July	1.0	2.7	1.9	0.4	0.9	2.8	1.8	0.5
Vascular rating								
40 cm	0.8	2.8	1.9	0.4	1.1	2.8	1.9	0.4
base	1.8	3.7	3.0	0.4	1.7	3.6	2.7	0.4
Lint yield, kg/ha	810	1930	1306	273	790	2010	1773	274
Reduction in lint yield, kg/ha†	-365	820	200	252	-410	795	138	263

† Difference between the mean lint yield of each line at two wilt-free locations and its lint yield at Dorot.

Table 3. Coefficients of correlation, r, between methods of grading for Verticillium wilt symptoms in two experiments at Dorot in 1972.

Wilt grading systems	Foliar rating 28 July	Vascular rating (40 cm)	Vascular rating (base)
	Exp. A		
Foliar rating, 11 July	0.71**	0.20	0.36**
Foliar rating, 28 July		0.18	0.51**
Vascular rating, 40 cm			0.47**
	Exp. B		
Foliar rating, 11 July	0.83**	0.32**	0.63**
Foliar rating, 28 July		0.41**	0.72**
Vascular rating, 40 cm			0.66**

** Significant at the 0.01 probability level.

due to differences between field plots. The regression coefficients indicated that each grade of foliar rating was associated on the average with a decrease of 369 kg/ha of lint yield. This is 32% of the total range for lint yield encountered in Exp. A and B (Table 2). Vascular discoloration at the base of the stem was also significantly correlated with lint yield explaining 25 to 29% of its variation. Vascular discoloration at 40 cm above ground level had a much lower correlation (nonsignificant in Exp. A) with lint yield and explained only 6% or less of its variation.

Reduction of lint yield at Dorot compared to the wilt-free locations was also significantly correlated with foliar symptoms, which explained 32 to 45% of the variation in the reduction. Each grade of foliar rating was associated on the average with a reduction of 359 kg/ha of lint yield. This is 30% of the total range for reduction in lint yield encountered in these experiments (Table 2). Vascular discoloration at the base of the stem explained 28 to 32% of the variation in lint yield reduction. Vascular discoloration at a height of 40 cm explained only 9% or less of this variation; and again, the correlation coefficient was not significant in Exp. A. Variation in lint yield reduction could also be caused by genotype by environment interactions not associated with wilt tolerance, as well as by random environmental effects.

The ranges and standard deviations of the grades (Table 2) were of approximately the same magnitude for all methods used to rate wilt symptoms. Coefficients of linear correlation between these methods are given in Table 3. The results of the foliar rating on both dates were highly correlated, and they were also significantly correlated with the vascular rating at the base of the stem. Vascular rating at a height of 40 cm was poorly correlated with the foliar ratings, the correlation coefficient being significant only in Exp. B.

DISCUSSION

The prevalent strain of Verticillium in Israel has not been defined, but observations indicate that it is a relatively mild strain somewhat less virulent than SS4 of the western USA.

Our preliminary (unpublished) result indicated that differences between tolerant and susceptible cultivars could be discerned most clearly when the infected plants were grown at a 27/22 C day/night temperature. Barrow (1) obtained good differentiation between cultivars inoculated with a mild isolate of Verticillium when the plants were grown at 25 C, whereas no symptoms developed at 35 C and at 18 C symptoms were equally severe on tolerant and susceptible cultivars. Bell and Presley (5) found that the expected reaction of tolerant and susceptible cultivars was most clearly expressed within a temperature range of 25 to 29 C, whereas all cultivars appeared to be resistant at 32 C. They reported that rates of conidial production were greatest at 20 to 25 C, while rates of phytoalexin synthesis in cotton plants were maximum at 27.5 to 35 C.

Our preliminary field experiment indicated that differences between mature plants of tolerant and susceptible cultivars could be detected most clearly in Israel after the middle of July when most of the bolls had set. Blooming usually starts in the Negev region at the end of June, and most of the final yield consists of bolls set from flowers of the first three weeks of the blooming period. Temperatures in July and August in that region are 17 to 19 C minimum, 30 to 33 C maximum, and the mean daily temperatures range from 24 to 26 C. These temperatures are in the range in which the expected tolerance reactions of cotton to mild Verticillium strains are usually observed. Bassett (3) reported that in the San Joaquin Valley of California, fewer Verticillium symptoms were observed on cotton plants and the correlation between those symptoms and lint yield was lower in June and July than in August and September. This was explained by the higher temperatures during July in that region which usually suppress the disease symptoms appearing earlier in May or June before the onset of flowering. Our results suggest that in addition to suitable temperature conditions a heavy load of developing bolls is necessary for effective differentiation between tolerant and susceptible cultivars.

The most important aspect of Verticillium tolerance from the cotton breeder's viewpoint is the ability of the plant to set a full load of bolls and to produce high yields in fields infested with Verticillium. However, the breeder must usually rely on visual symptoms for screening and selection of individual plants or breeding lines. The most efficient method of visual rating would be that which is most highly correlated with lint yield in infested fields. The results of our 1972 experiments indicate that foliar symptoms are more suitable than vascular symptoms for this purpose. Vascular discoloration at the base of the stem was better correlated with yield reduction and with foliar symptoms in field experiments than vascular discoloration at a height of 40 cm.

Therefore, we concluded that screening by observing vascular discoloration in sections of the stem is less effective (and incidentally more time-consuming) than by observing foliar symptoms. Similar conclusions were reached by Bassett (3) who found no significant correlations between postharvest vascular ratings and cotton yield. Significant correlations between disease ratings based on foliar symptoms and cotton yield have been reported by several investigators (3, 8, 9). These conclusions are supported by the findings of Garber and Houston (6) that resistance of cotton to Verticillium is not primarily related to the ability of the roots to withstand invasion of the organism, but rather to the ability to suppress colonization throughout the plant once it does enter the vascular system.

Our results indicate that cotton plants or breeding lines may be successfully screened for their tolerance to Verticillium by observing foliar disease symptoms. The similar results of both dates (11 and 28 July) indicate that this screening can be carried out within a period of at least three weeks. The following conditions appear necessary for most efficient screening: (a) observations should be made at the right stage of plant development, i.e., after the middle of the flowering period when the plants are carrying many developing bolls; (b) average daily temperatures should be in the range of 24 to 26 C which is reported by several authors (1, 5) as optimum for the expression of differential tolerance; (c) and the field should be fairly uniformly infested with Verticillium.

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