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Relationship Between Field and Greenhouse Ratings for Tolerance to Verticillium Wilt on Cotton[1]

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ABSTRACT

Greenhouse evaluations of tolerance to Verticillium wilt, caused by *Verticillium dahliae* Kleb., in cotton, *Gossypium hirsutum* L., have been used in the past; however the relationship between field and greenhouse evaluations for the disease is not clear. In the present study, 48 cultivars and breeding lines of *G. hirsutum* were evaluated following induced infection by stem or root inoculation in the greenhouse and natural infection under field conditions (Exeter loam soil; Fine-loamy, mixed, thermic Typic Durixeralfs). Highly significant differences were observed among genotypes in both greenhouse and field experiments, and broad-sense heritability estimates were comparable, 0.70 and 0.74 for field and greenhouse, respectively. There was good agreement between field ratings and greenhouse reactions following stem inoculation; phenotypic and genotypic correlations were 0.72 and 0.86, respectively. There was essentially no relationship between field ratings and greenhouse reactions following root inoculation. Selection for wilt tolerance on the basis of field evaluations may result in later maturing plants and reduced lint yield since these characters appear to be associated with tolerance. However, greenhouse screening, which uses young plants, may avoid these correlated responses to selection. Greenhouse evaluation of Verticillium wilt tolerance may be used with confidence in selection programs for disease tolerance.

***Additional index words:* *Gossypium hirsutum* L., Earliness, *Verticillium dahliae* Kleb., Inoculation techniques, Temperature effects.**

VERTICILLIUM wilt, caused by the soilborne fungus *Verticillium dahliae* Kleb., is one of the most destructive diseases of cotton (*Gossypium hirsutum* L.). Damage is particularly severe in more temperate areas of cotton production, such as the San Joaquin Valley of California or the high plains of Texas. In California the disease is currently managed by the use of tolerant Acala cotton cultivars, crop rotation, and irrigation practices. However, highly virulent strains of *V. dahliae* are widespread and frequently cause considerable reduction of lint yield in fields planted to tolerant cultivars (2).

Breeding for tolerance to Verticillium wilt typically involves field selection based on foliar or vascular symptoms. However, disease expression is complicated by strong environmental effects, particularly temperature (12), and by associations of wilt tolerance with increased vegetative growth, late maturity, and reduced boll load (4,10). Several workers have previously correlated cotton yields with various methods of field evaluation for Verticillium wilt tolerance (3,10,13).

In view of these problems, greenhouse evaluation for disease tolerance may be a preferred alternative to field screening. Greenhouse evaluation of cotton has been used extensively in the past (1,15,17) because it allows more precise environmental control. Also, since young plants are used, a large number of individuals can be rapidly evaluated in a relatively small area. However, the relationship between young and mature plant tolerance to Verticillium wilt is not clear.

The objectives of this study were to compare two common inoculation procedures for *V. dahliae* on young plants, and to determine the relationship between greenhouse reactions and the reactions of naturally infected mature plants in the field. We also examined the associations between plant maturity, lint yield, and wilt tolerance.

MATERIALS AND METHODS

The investigation consisted of three greenhouse experiments and a 2-year field study. The cotton cultivars and breeding lines chosen for evaluation were all upland cottons (*G. hirsutum* L.), and represented a range of tolerances to Verticillium wilt.

The pathogen, *V. dahliae*, was collected from field-infected plants near Strathmore, CA. Single-spore isolates were maintained on porcelain beads (9), and subsequently used to propagate the fungus on potato dextrose agar slants or in shake culture. The pathogenicity of each isolate was compared to a standard isolate, T-9 (provided by J.E. DeVay,

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Univ. of California, Davis). The disease reactions on three cultivars, 'Acala SJ-5', 'Acala 4-42', and 70-110, showed that all of the isolates tested had the ability to defoliate and were of comparable virulence to T-9. Twelve of these isolates were individually propagated and composited for the greenhouse studies. The density of spores was determined using a hemocytometer, and appropriate dilutions were made to give an inoculum density of 10^5 spores mL^{-1}. The same isolates and inoculum, density were used in each greenhouse experiment.

A split-plot design was used in the first greenhouse experiment to evaluate the efficacy of stem and root inoculation techniques and to determine whether the disease reaction (foliar symptoms) was consistent for the two methods of inoculation. Sixteen cotton genotypes were evaluated over five replications, each plot consisting of two seedlings in separate 10-cm plastic pots. The whole plots were the inoculation techniques, and the subplots were the genotypes.

Inoculations were made when the plants were four to five weeks old. Stem inoculations were made in the center of the cotyledonary node using *ca.* 0.1 mL of inoculum delivered from a 21 gauge hypodermic needle. Root inoculations were made by loosening the sides of the pot and pouring 200 mL of a spore suspension (10^5 spores mL^{-1}) around the roots. The greenhouse temperature ranged from 19° to 34° C; the average day and night temperatures were 23.4° and 27.0° C, respectively.

Evaluations for disease tolerance were made 3 weeks after stem inoculation and 6 weeks after root inoculation. The foliar symptoms took considerably longer to appear when plants were root inoculated. A plant that was completely defoliated was scored as 10, and lesser degrees of defoliation and leaf chlorosis or necrosis were rated relatively from 0 to 9.

The second greenhouse study included 48 cultivars and breeding lines that also were evaluated in a field study, and all seedlings were stem inoculated. The 48 genotypes were arranged in a randomized complete block design (RCBD), each plot consisting of four seedlings in a 7.6 L pot. This experiment was performed in two successive winters.

The field study included 48 cultivars and breeding lines which were evaluated at the California Planting Cotton Seed Distributors' research station near Strathmore, California. The location had a history of particularly severe Verticillium wilt disease and uniform, severe infection resulted without supplemental inoculation. Soil at the test site was an Exeter loam (fine-loamy, mixed, thermic Typic Durixeralfs). Isolations from diseased plants did not detect the presence of another wilt pathogen, *Fusarium oxysporum* f. sp. *vasinfectum*. Entries were planted in mid-April in 7.6 m rows spaced 1 m apart. The field entries were replicated twice in a RCBD and evaluated for two consecutive years (1982 and 1983). Disease ratings based on foliar symptoms were taken in mid- September. Plot ratings were made from 0 to 10, with 0 being a plot free of wilt symptoms. Visual ratings for earliness of plant maturity, based on relative numbers of open bolls, were made in both years. In 1983, earliness was also quantified as the earliness yield ratio: the ratio of weight of seed cotton harvested in an early picking (15 Sept) to total weight of seed cotton harvested. Five plants/plot were hand harvested at the two dates to determine this ratio; the total yield from both dates gave a measure of lint yield.

Effects due to replications and years were considered random and all other effects fixed in the individual and combined year analyses of variance and covariance. In the combined analyses of variance, the mean square for genotypes was tested against the pooled error term because the mean square for the genotype × year interaction was not significantly different from error in the analyses presented. Two types of correlations were calculated between traits of interest (8). Phenotypic correlations were calculated from the observed variance and covariance of entry means. Genotypic correlations were calculated by partitioning the variance and covariance for the traits into total genetic and environmental components using analyses of variance or analyses of covariance. Broad-sense heritability estimates for Verticillium wilt tolerance were calculated on an entry mean basis using variance components estimated from the analyses of variance combined over years.

RESULTS

Disease rating differences among cultivars and breeding lines were highly significant in all tests in the field and in the greenhouse. Means for the 16 genotypes which were common to all tests are presented in Table 1. The breeding line, S 5971, was among the most tolerant in both field and greenhouse tests. Ranking of cultivars was generally similar for ratings in the field and greenhouse.

In the analysis of variance for the split-plot design, the differences between inoculation methods and among genotypes were both highly significant ($P < 0.01$). The inoculation method × genotype interaction term was nonsignificant ($P > 0.05$), indicating that the response of cultivars and breeding lines was similar to each of the inoculation methods. Ratings of stem inoculated plants more closely approximated field scores than did ratings of root inoculated plants. Phenotypic correlations (rp) and genotypic correlations (rg) for the 16 cultivars and breeding lines using stem inoculation procedures were 0.60 and 0.70, respectively; there was no relationship between field scores and greenhouse scores of root-inoculated material ($rp = -0.06$).

In subsequent greenhouse and corresponding field experiments, differences among genotypes were

Table 1. Mean field and greenhouse ratings for Verticillium wilt tolerance and maturity of 16 cotton cultivars and breeding lines over 2 years.

Genotype	Maturity†	Disease ratings‡ Field	Greenhouse
S 5971	L	0.25	4.19
T 8038	ML	0.50	5.75
'Acala SJ-5'	ML	1.00	5.57
'Acala SJ C-1'	ML	1.25	5.38
'Lambright GL-4'	M	1.35	6.13
'Acala 4-42'	ML	2.25	6.07
6024 Arizona	ML	2.25	6.63
P 5981	M	2.25	7.38
'Deltapine 30'	M	2.50	7.00
T 4445	ML	2.70	5.25
'Acala 1517 E2'	M	3.00	5.82
'Deltapine 70'	M	4.75	7.94
Glandless Acala	VE	5.50	7.88
70-110	E	5.75	8.00
CHRP	E	6.00	8.25
Erg 7-8	E	6.00	9.19
LSD (0.05)		1.72	2.15

† VE = Very Early; ML = Medium Late, etc.
‡ Disease ratings, 0 to 10, with 0 = no symptoms.

highly significant (Table 2). Also, there were no genotype × year interactions, indicating that the lines ranked consistently from year-to-year. Broad-sense heritability estimates for disease tolerance were comparable in the two tests (Table 2), and the phenotypic correlations indicated a close association between field and greenhouse scores for disease tolerance (Table 3).

Evaluations for earliness of plant maturity indicated that there was considerable variability for this trait among the lines tested. The visual ratings for earliness were very closely related to evaluations based on the earliness yield ratio (Table 3). The combined analysis for visual ratings of earliness indicated highly significant differences among genotypes and no significant genotype × year interaction. Earliness and disease susceptibility showed a close relationship, with early varieties more susceptible to the disease (Table 1). Lint yield also tended to be associated with susceptibility; high yielding lines were generally more susceptible to Verticillium wilt.

DISCUSSION

A number of techniques have been used to evaluate host-plant reactions to *V. dahliae* in the field and greenhouse. Evaluations of mature plants have indicated that foliar symptoms explained 31 to 45% of the variation in lint yield among cotton cultivars and breeding lines in a wilt-infested field (10). This was slightly more than the 25 to 29% variation in lint yield attributable to vascular discoloration at the base of the stem.

Various methods of evaluation and seedling inoculation of cotton with *V. dahliae* have been reviewed (16). Erwin and coworkers (7) compared the stem puncture method with root dipping in spores and found that the incidence of diseased plants was greater with the stem puncture method. Moreover, root dipping induced problems with physiological stunting, even in the absence of the disease. In the present study, the root inoculation method used was a variation of this technique where the plants were not actually removed from the pots for inoculation. However, these procedures still did not show an advantage for root inoculation. Not only was the procedure itself more difficult to implement, but the results were highly variable and inconsistent with disease incidence under field conditions.

Table 2. Analyses of variance combined over 2 years, and broad-sense heritabilities of field and greenhouse (GH) ratings for Verticillium wilt tolerance.

	df		Mean squares	
Source	Field	GH	Field	GH
Years	1	1	4.57	600.00
Reps/years	2	6	7.98	37.63
Genotypes	47	47	8.51**	31.65**
Genotype × Year	47	47	1.62	5.63
Error	94	282	1.49	4.83
Heritability			0.70	0.74

** Significant at the 0.01 probability level.

Schnathorst and Cooper (14) did not find any consistent relationship between reactions to Verticillium wilt in the field and greenhouse. They used stem inoculation with a mild strain (designated SS-4) of *V. dahliae* in greenhouse tests, whereas a defoliating strain (T-1) predominated in field evaluations. The SS-4 strain was used in the greenhouse because the T-1 strain killed all cotton seedlings even though they may have had some tolerance to T-1 under field conditions.

Perhaps the most important difference between the present study and Schnathorst and Cooper's (14) study is the temperature at which the greenhouse plants were evaluated. Schnathorst and Cooper made their evaluations of wilt tolerance at 21°to24°C. It has been reported that all *Gossypium* spp. tend to be susceptible to Verticillium wilt at 22°to24°C, and resistant at 30°to32°C (5). In the present study, we chose a temperature range, generally 23°to30°C, that would allow differences in phenotypic expression of wilt tolerance.

Recent work suggests that there is a continuum of aggressiveness in strains of the pathogen, and that this is expressed under varying host selection pressure (1,2). Considering this observation, a random sample of several field isolates, as opposed to one, should be a more appropriate test of tolerance among the genotypes. Using 12 isolates and the stem inoculation method, we were able to differentiate tolerance levels to Verticillium wilt among genotypes. The reactions in the greenhouse were closely paralleled by disease reactions in the field. Broad-sense heritability estimates under field and greenhouse situations were 0.70 and 0.74, respectively, indicating that selection for wilt tolerance under either situation should be equally effective.

The genotype × year interaction was nonsignificant in either greenhouse or field tests. The incidence of Verticillium wilt infection, as measured by foliar symptom development, is affected by a number of factors including: cropping history, fertilization practices, cultivars of cotton, fungal pathotypes pres-

Table 3. Phenotypic (*rp*) and genotypic (*rg*) correlation coefficients between field Verticillium wilt ratings and associated agronomic traits.

	Greenhouse vert score		Earliness rating		Earliness yield ratio		Yield	
	rp	*rg*	*rp*	*rg*	*rp*	*rg*	*rp*	*rg*
Field vert score	0.72**	0.86	0.69**	0.89	0.67**	0.82	0.26	0.36
Greenhouse vert score			0.51**	0.73	0.44**	0.53	0.15	0.17
Earliness rating					0.83**	1.00	0.29	0.48
Earliness yield ratio							0.51**	0.62

** Significant at the 0.01 probability level.

ent in one field versus another, and daily temperature (11,12). The present study was done at a single location and isolates of *V. dahliae* were taken from this location for greenhouse inoculations. This uniformity, especially for pathotypes of *V. dahliae* (1), may account for the low interaction terms.

Tolerance to Verticillium wilt is frequently associated with indeterminate growth, late maturity, and reduced lint yield (4). The same associations were demonstrated in the present study, although there was no control to evaluate these traits on Verticillium-free land. The simple correlation of greenhouse ratings for disease tolerance vs earliness was significant, but lower than field ratings vs earliness (Table 3). The correlation between greenhouse score and earliness may be a reflection of the close relationship between greenhouse score and field score which was also closely related to earliness. Tolerance to Verticillium wilt in young plants would not be expected to be influenced by earliness. Perhaps greenhouse evaluations of tolerance effectively avoid some of the changes due to earliness which result from field evaluation for wilt tolerance.

Plant maturity is also associated with resistance to the potato "early dying" disease caused by *V. dahliae.* Corsini et al. (6) showed that greenhouse tests were effective in differentiating apparent field resistance due to late maturity and true resistance. Colonization by *V. dahliae* under controlled greenhouse conditions appeared to eliminate the maturity problem since maturity was not correlated with *V. dahliae* colonization under such tests.

The principle conclusion from the present study is that there is good agreement between cultivar and breeding line reactions following stem inoculation in the greenhouse and reactions under severe natural epidemic conditions of Verticillium wilt in the field. This agreement indicates that seedling inoculation and evaluation in the greenhouse may be used with confidence in certain research projects or in breeding programs for tolerance to the disease.

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