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Correlation of *Fusarium* Wilt of Cotton in the Field and Greenhouse[1]

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ABSTRACT

Cultivar expression of cotton (*Gossypium hirsutum* L.) to *Fusarium oxysporum* Schlecht f. *vasinfectum* (Atk.) Snyd. & Hans. was studied under field conditions where nematodes were present and under greenhouse conditions where only the wilt fungus was artificially introduced into the plant.

In greenhouse studies, seedlings were artificially inoculated with either mixtures or individual wilt isolates in the absence of nematodes. When a highly-virulent isolate of fusarium was used for greenhouse inoculations, results were highly correlated with those obtained under field conditions where nematodes and wilt were both present. Isolates of fusarium obtained from resistant or susceptible varieties were not always of high or low virulence, respectively. Therefore inoculum of known high virulence must be used in greenhouse tests for symptom expression to be correlated with that obtained under field conditions.

***Additional index words:* *Gossypium hirsutum* L., *Fusarium oxysporum* f. *vasinfectum*, Nematodes.**

SELECTIONS for resistance to fusarium wilt [*Fusarium oxysporum* Schlecht f. *vasinfectum* (Atk.) Snyd. & Hans.] in cotton (*Gossypium hirsutum* L.) have been made for many years. Probably some of the first work in upland cultivars was conducted by Orton in 1899 (12). Although selection for fusarium-wilt-resistant cultivars has been continued over the years, the most resistant cultivars commercially available do not always exhibit the best yield or fiber properties.

Many fusarium-wilt-resistant cultivars may lack commercial acceptability for many reasons. First, unfavorable genetic linkage for fusarium-wilt resistance and high yield and quality factors may exist. Also, many lines have been selected for high-quality fiber characteristics and yielding ability based on results from one test and for fusarium-wilt resistance based on results from a different test. The final cultivar released may be a compromise of the best from both tests. Finally, if testing facilities are not available, adequate selection pressure for disease resistance cannot be applied during all phases of a breeding program.

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Several greenhouse procedures have been developed to evaluate cotton for resistance to fusarium wilt (1, 3, 11, 14). However many breeders do not use any of these procedures. Instead, they base most selections on field performance of materials grown either in individual wilt tests or in the regional fusarium wilt screening test (6, 8, 9, 10). This latter test, available to all cotton breeders, has been conducted at Tallassee, Ala., for many years. High natural infestation of this test site allows excellent symptom expression and differentiation between fusarium wilt susceptible and resistant materials. However space limits the number of entries tested during the growing season. Therefore, all materials may not be evaluated as extensively as needed. Furthermore in field tests, nematodes (mainly Meloidogyne, Belonolaimus, and Criconemoides spp.) are often present and could influence the results in tests for fusarium wilt resistance.

With the procedures described by Bugbee and Presley (3) and later modified by Bugbee and Sappenfield (4), more materials could be evaluated quicker in the greenhouse than in field tests. At 4 weeks after planting, plants are artificially stem-inoculated with the fusarium fungus and differentiated on symptom expression 4 weeks later. Possibly some researchers hesitate to use a laboratory or greenhouse test because they lack knowledge about the relative performance after natural infection under field conditions vs. artificial inoculation under greenhouse conditions.

The present study was conducted i) to determine how results obtained under field conditions compare with those obtained after artificial inoculation with the wilt fungus in the greenhouse and ii) to compare how effective various wilt isolates are for screening for fusarium resistance in the greenhouse.

MATERIALS AND METHODS

Twenty-four cotton cultivars were evaluated for fusarium-wilt resistance in the greenhouse after they had been inoculated artificially with a suspension of spores. All entries were evaluated for wilt resistance under field conditions at Tallassee, Ala., during 1971 (8). Some entries were evaluated also in the regional fusarium wilt screening test at Tallassee, during 1970, 1972, and 1973 (6, 9, 10).

Eight greenhouse tests were conducted. Inoculum used for these tests contained 2×10^6 microconidia/ml. Sources and identification of isolates are as follows:

Table 1. Percentage of cotton plants infected with *Fusarium* wilt in field and greenhouse tests.

Entry designation	Regional test 1970	1971	1972	1973	Greenhouse test (isolate) 72-1 (B, D)	72-2 (C, E, F, G, H, I)	72-3 (A, J, K, L, M)	72-4 (K)	72-5 (A)	72-6 (L)	72-7 (M)	72-8 (J)	Weighted mean
Hy-Bee 200	87	82	44	13	45	23	45	25	28	45	13	81	44
Hy-Bee 100	56	58	35	38	69	52	68	33	59	57	0	25	46
McNair 511	24	37	17	31	16	35	18	34	6	22	6	11	21
Deltapine 45A	58	72	10	30	22	39	26	30	31	19	25	13	31
Susceptible Rowden	97	92	67	70	75	94	73	45	89	73	26	65	72
Coker 310	46	71	10	47	44	48	37	54	20	56	12	20	39
Deltapine 16	71	58	26	15	61	51	56	33	40	75	10	64	47
Susceptible Redleaf	93	89	78	67	86	91	81	64	100	90	21	68	77
Coker 417	59	49	30	36	49	56	57	42	40	67	19	71	48
Auburn 56	23	18	10	26	38	56	33	38	20	50	19	22	29
Dixie King II	12	45	16	21	41	38	47	10	59	36	11	53	35
Stoneville 603	53	50	20	34	43	21	39	63	10	44	6	25	34
Hancock	92	94		84	43	80	81	40	88	17	25	90	65
Tamcot SP21	52	58		57	47	38	61	88	45	65	23	31	50
Tamcot SP37	29	64		64	58	63	57	38	69	50	32	22	49
Auburn M	18	39	27		15	39	20	31	28	16	22	4	24
Delcott 277		38	12	21	34	42	42	55	45	29	29	32	34
McNair 1032 B	58	46	21		29	42	34	48	24	45	11	17	34
McNair 210		27	29	43	52	55	55	53	45	57	0	65	42
DPL-SL		96		65	70	91	26	75	86	44	16	43	62
DPL-6225	37	33			23	48	40	50	29	47	61	28	36
MoDel	48	65			48	60	42	33	25	47	15	55	43
Coker 310-70903	96	74			44	58	40	42	30	50	11	28	47
Tamcot SP23	55	60			45	40	76	88	75	38	27	77	54

Table 2. Correlation of wilt expression of cottons grown under field conditions of high levels of both *Fusarium* and nematodes and greenhouse conditions where plants were inoculated with single or mixtures of *Fusarium* isolates.

	Regional test 1972	1971	1970	Greenhouse test (isolate) 72-1 (B, D)	72-2 (C, E, F, G, H, I)	72-3 (A, J, K, L, M)	72-4 (K)	72-5 (A)	72-6 (L)	72-7 (M)	72-8 (J)
Test											
1973	0.66**	0.60**	0.39	0.49*	0.77**	0.53*	0.43	0.73**	0.16	0.39	0.22
1972		0.67**	0.76**	0.76**	0.71**	0.77**	0.19	0.79**	0.67**	0.15	0.61*
1971			0.81**	0.57**	0.56**	0.39	0.11	0.60**	0.19	0.01	0.43*
1970				0.55**	0.48*	0.56**	0.08	0.44*	0.41	-0.07	0.58**
Isolate											
B, D					0.68**	0.67**	0.26	0.71**	0.76**	-0.20	0.51*
C, E, F, G, H, I						0.44*	-0.15	0.75**	0.36	0.16	0.36
A, J, K, L, M							0.28	0.73**	0.51*	0.09	0.71**
K								0.34	0.24	0.16	0.12
A									0.29	0.20	0.57**
L										-0.10	0.32
M											-0.01
df	15	23	20	23	23	23	23	23	23	23	23

* ** Test results significantly correlated at the 0.05 and 0.01 levels of probability, respectively.

Isolate	Designation	Description
3.5024-2	A	Isolate from diseased 'Auburn 56' plant from the 1966 regional wilt test.
R93.9	B	1969 reisolation from greenhouse materials infected with isolate 3.5024-2
A56-9	C	Isolates from infected Auburn 56 plants from the 1970 regional wilt test
A56-13	D	
A56-25	E	
A56-82	F	
ST. 7A-172	G	Isolates from infected 'Stoneville 7A' plants from the 1970 regional wilt test
ST. 7A-202	H	
ST. 7A-261	I	
57	J	Isolates from infected plants collected throughout Alabama in 1971
66	K	
71	L	
MO3-2	M	

Inoculum was produced by growing isolates in Czapek's solution as shake cultures at 27 C. After 7 days, single cultures were mixed in a blender, spores were counted using a hemocytometer, and then cultures were adjusted to the desired concentration with sterilized distilled water. Inoculum containing more than one wilt isolate was prepared by mixing equal parts (V:V) of the desired isolates after adjustment to the proper spore concentration.

Seeds of materials evaluated in greenhouse tests were hot-water treated at 80 C for 90 sec and then placed in a germinator at 30 C. After about 24 hours, radicles had emerged to a length of 6 to 13 mm. These germinated seeds were then planted in rows on greenhouse benches containing Wickham sandy loam which had been treated previously with methyl bromide. This soil was obtained from the Tallassee field where the regional fusarium wilt screening tests were conducted.

Germinated seeds were placed in preformed holes in the soil. Holes were about 6 mm × 32 mm deep. Immediately after planting, the seeds were covered with soil and watered. Plants were spaced about 51 mm within the row. Distance between rows was 152 mm. Although air temperature was set to be maintained from 24 to 29 C, occasionally air temperature exceeded 38 C during the heat of the day.

Four weeks after planting, plants were stem-inoculated (two inoculations/plant) by the technique described by Bugbee and Presley (3), at about 13 mm above the soil line. Inoculum used for the different tests was as follows:

Test	Inoculum
72-1	Equal parts of isolates A56-13 and R93.9
72-2	Equal parts of isolates ST. 7A-172, -202, and -261 and A56-9, -25, and -82
72-3	Equal parts of isolates 66, 3.5024-2, 71, MO3-2, and 57
72-4	Isolate 66
72-5	Isolate 3.5024-2
72-6	Isolate 71
72-7	Isolate MO3-2
72-8	Isolate 57

After inoculation, wilted plants were removed at weekly intervals. Remaining healthy plants were counted at 4 weeks after inoculation. Wilting percentages were then calculated. Each test had been designed as a randomized complete block with four replications; 10 plants were evaluated/replication.

In field tests, 200 seeds were planted/9m row with 1 m between rows. Seed depth was about 32 to 38 mm. Four replications of each entry were evaluated using a systematically randomized test design.

In 1970, the first wilt counts in the field test were made at 6 weeks after planting. Five more wilt counts were made at about 2-week intervals and final healthy plants were counted at 122 days after planting. During 1971, the first wilt counts

were made at 9 weeks after planting, followed by four more counts at about 2-week intervals, and final healthy plants were counted at 132 days after planting. In 1972, live-plant counts were made at 59 days and again at 124 days after planting. During 1973, live plants were counted first at 44 days, wilted plants removed at 65 days, and final live plants counted at 134 days after planting. Differences between first and final live-plant counts during 1972 and 1973 were attributed to wilt losses. Percentage plants/plot and mean wilting percentages for a given entry were determined. Then all possible correlations between results from all tests were calculated.

RESULTS AND DISCUSSION

Isolates

Mean wilting percentages varied greatly over tests; however such differences have been observed previously (7, 13). In general, the susceptible checks ('Rowden' and 'Redleaf') wilted most; whereas 'McNair 511,' Auburn 56, and 'Auburn M' were highly resistant (Table 1). The overall severity of wilting under greenhouse conditions was not as great as that in field tests during 1970 and 1971. In general, plants wilted most under greenhouse conditions in tests 72-2 and 72-3. In both of these tests, in which mixtures of isolates C, E, F, G, H, and I and A, J, K, L, and M were used, wilting was significantly greater than that in the test involving only isolates M (72-7). However wilting in tests 72-2 and 72-3 did not differ greatly from that in tests 72-1 and 72-5. Isolates B and D were used in test 72-1, whereas only the highly virulent isolate A (3.5024-2) was used in test 72-5. Comparisons among the amounts of wilting when single wilt isolates were used as inoculum showed differences in virulence between isolates.

When mixtures of highly virulent wilt isolates were used as inoculum, fusarium-wilt susceptibilities of cultivars grown under greenhouse conditions differed like those previously reported for field results (7). However resistances of cultivars did not always differ in greenhouse tests when pathogenic, but not highly-virulent isolates, were used as inoculum. Where some such isolates were used, relative cultivar differences were reversed. Tests could have differed also because nematodes were absent in greenhouse tests, but present in all field tests. Some materials evaluated may have been somewhat resistant to nematodes, whereas others may not. Thus nematode-susceptible cultivars may have shown greater wilt damage in the field because, as other workers have shown, plants wilt more when nematodes and fusarium are both present (4, 5, 13). Therefore with these greenhouse techniques, mixtures of wilt isolates or highly virulent isolates must be used to get adequate wilt differentiation.

Correlation of Field and Greenhouse Results

With one exception, results from greenhouse tests where inoculum consisted of a mixture of wilt isolates (tests 72-1, -2, -3) were positively correlated with field results (Table 2). In addition, results among these three tests were correlated highly. However results from greenhouse test 72-3 were not significantly correlated with those obtained in the 1971 regional test. When individual wilt isolates were used as the source of greenhouse inoculum, only the highly virulent isolate 3.5024-2, A (test 72-5), incited wilting comparable to and correlated with that obtained in field tests or greenhouse tests involving mixtures of wilt isolates as inoculum.

Using the root inoculation method of Wickens (14), Brown (2) found resistance of three cotton strains selected in the greenhouse for wilt resistance after artificial inoculation comparable to field tests. In addition, he found no breakdown in wilt resistance in the presence of root-knot nematodes at infestation levels experienced in his field tests. However he stated that higher levels of nematode infestation might affect wilt resistance. Work by Bugbee and Sappenfield (5) showed that the true disease resistance might be obscured when plants were simultaneously infected with more than one pathogen.

Because high nematode infestation occurred in all regional tests, symptom expression may have been greater because of the nematodes as well as the wilt organism. As a result, a selection might appear susceptible in the field but resistant in greenhouse tests, where nematodes were not present. Thus lack of a higher correlation between field and greenhouse results might have been caused by the difference in organisms present.

The data show that when cotton was needle-inoculated with fusarium using two injections/plant (4) and grown under greenhouse conditions, it resisted wilt as if it had been grown in the field. Because this expression was also correlated with that observed under field conditions of wilt and nematodes, use of such a screening procedure would allow a rapid evaluation of many more materials than can be field-tested. However researchers should select isolates of the wilt organism cautiously. Inoculum consisting of a mixture of virulent isolates probably will incite wilt development and symptom expression similar to that expected in the field.

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