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Cotton Resistance to the Root Knot–Fusarium Wilt Complex. I. Relation to Fusarium Wilt Resistance and its Implications on Breeding for Resistance[1]

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ABSTRACT

Fusarium wilt disease (FW) caused by *Fusarium oxysporum* Schlect f. *vasinfectum* [(Atk.) Synd. & Hans.] and root-knot nematodes (RKN) [*Meloidogyne incognita* (Kofoid and White) Chitwood] cause a major disease complex affecting cotton (*Gossypium hirsutum* L.). The purpose of this study was to determine the efficacy of using resistance to FW in the vascular system of plants grown in a greenhouse as a criterion for selecting cotton plants that are resistant to FW in the RKN-FW complex as it occurs in the field. Eighteen cotton cultivars and breeding lines were inoculated in a greenhouse by injecting spores of the FW fungus directly into stems. Incidence of FW ranged from 29 to 82%. The FW percentages of the 18 cotton cultivars and lines in a field infested with both the fungus and RKN ranged from 3 to 99%. Results indicated that stem vascular resistance to FW was probably independent of any mechanism of resistance that might have prevented fungal invasion of the vascular system and that differences in vascular resistance were probably genetically controlled. Vascular resistance observed in the greenhouse failed to discriminate between differences in FW resistance among several cottons when grown in the field. This and other evidence presented indicated that a low probability of success would be expected for developing cotton cultivars with high field resistance to FW in the RKN-FW complex by either selecting for vascular resistance expressed in plants grown in a greenhouse or through field selection by present techniques. Field selection for many decades had resulted in development of moderately resistant cultivars but had not been successful in developing highly FW-resistant cultivars. Further progress by the field selection method may be possible in the future, if genes for higher resistance, such as in breeding line Auburn 623 RNR, are used and more precise screening techniques can be developed.

Additional index words: **Cotton breeding, Genetics of resistance, Mechanisms of resistance, Vascular wilt, *Gossypium hirsutum* L., *Meloidogyne incognita*, (Kofoid & White) Chitwood, *Fusarium oxysporum* f. *vasinfectum* [(Atk) Synd. & Hans.].**

A VASCULAR wilt fungus, *Fusarium oxysporum* Schlect f. *vasinfectum* (Atk.) Snyd. & Hans., causes severe fusarium wilt disease (FW) of upland cotton (*Gossypium hirsutum* L.) when predisposing agents such as root-knot nematodes [*Meloidogyne incognita* (Kofoid & White) Chitwood] (RKN) are present. The FW disease usually is severe in the field only in the presence of nematodes, which are usually RKN. The complex of disease symptoms expressed in the field in response to interaction of the fungus with RKN (RKN-FW disease complex) makes it difficult to distinguish effects of FW resistance from those of RKN resistance when selecting plants for breeding purposes.

Injecting FW spores directly into the vascular system, by procedures described by Bugbee and Presley (7) and Bugbee and Sappenfield (8), appeared to offer the best approach to assessing FW resistance in the absence of RKN. Negative aspects of these procedures are the bypassing of possible resistance mechanisms in the rhizosphere and roots. These mechanisms may include: root exudates that are antibiotic to vascular-wilt fungi (3); occurrence in the rhizosphere of microflora allelopathic to vascular fungi (5); and barriers to fungal penetration, colonization, and proliferation in the root epidermis, cortex, endodermis, or vascular

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system (xylem) (13). However, measurement and quantification of these mechanisms under field conditions would probably be difficult because some or all of them may be confounded by RKN effects.

On the positive side, vascular inoculation by Bugbee's procedures probably assesses differences in resistance to FW development in the vascular system. Talboys (13) divided mechanisms of resistance to vascular-wilt fungi into prevascular and vascular phases according to whether they acted before or after a fungus invaded the vascular system. Several possible mechanisms of vascular resistance to vascular-wilt fungi include: degradation of fungal cell walls or detoxification of fungal metabolites, enzymes, and toxins by host enzymes (13); selective support, in the vascular system of resistant cotton, of microflora antibiotic to vascular wilt fungi (5); absence of receptor sites on host cell membranes for fungal toxins or blockage of such sites by host enzymes (9); and processes in the vascular system that provide physical barriers to systemic infection (10). These mechanisms might act independently, together, or in succession.

Beckman et al. (2) found no structural differences in the vascular systems of cotton that could account for differences in resistance. They reported that speed of the hosts reaction against the fungus appeared to be the crucial difference between resistance and susceptibility, with resistant cotton plants responding much more rapidly than susceptible ones. Bugbee (6) reported that phytoalexins were probably involved in slowing or stopping fungal growth in the vascular system.

The purposes of this study were to determine FW resistance in the vascular system of plants grown in a greenhouse in a group of cotton cultivars and advanced breeding lines, and to relate FW resistance in the vascular system with resistance to FW in the RKN-FW complex as observed in plants grown in the field. These studies will aid in determining the efficacy of using vascular FW resistance in selecting cotton for resistance to FW of the RKN-FW complex in the field.

MATERIALS AND METHODS

Greenhouse Tests for FW Resistance. Eighteen cultivars and breeding lines were evaluated for FW resistance in a series of three greenhouse tests. Cultivars were: Bayou, Coker 100A (Cok 100), Coker 201, (Cok 201), Coker 310 (Cok 310), Delcot 277 (Del 277), Deltapine 16 (DPL 16), Deltapine Smoothleaf (DPL SL), Dixie King II (Dix K), Empire WR 61 (Emp 61), McNair 511 (McN 511), Model, Rowden, and Stoneville 213 (Stv 213). Rowden was highly susceptible to both FW and RKN. Breeding lines were Auburn 623 RNR (Aub 623), a line with the highest RKN and FW resistance known in *Gossypium* sp.; Auburn 56 line (Aub 56L), an inbred line of Auburn 56 that was unselected for RKN and FW resistance; Auburn BR-1 (Aub BR-1), a line selected for FW resistance in the field; Clevewilt-6 (Clevew), a line with moderate RKN and FW resistance; and M-8, a doubled haploid derived from the cultivar Deltapine 14, which was highly susceptible to both FW and RKN.

Each test was arranged in a randomized complete block design with six replications. Tests were conducted in greenhouse benches filled 150 mm deep with a sandy silt loam soil. Experimental plots were 0.15 m wide × 0.5 m long rows in the greenhouse benches, with plants spaced 50 mm apart in rows. By methods previously reported (7), inoculum of six different isolates of *F. oxysporium* f. *vasinfectum* was produced in flasks of Czapek's solution on a waterbath shaker maintained at 27 ± 5°C for 7 days after inoculation. Four weeks after planting, equal quantities from each isolate were mixed to give 2×10^6 microconidia/mL, and this inoculum was used to inoculate plant stems following the techniques described by Bugbee and Presley (7). Inoculum was injected into opposite sides of each stem about 13 mm above the soil line.

Plants showing foliar symptoms of FW were counted and removed each week. Foliar symptoms included discrete areas of the leaf that had turned yellow or brown, leaf wilting, and defoliation. Plants exhibiting foliar symptoms also were examined for vascular symptoms of the disease as indicated by dark brown or black vascular discoloration. Healthy plants remaining 4 weeks after inoculation were counted. Percentages of plants with symptoms were calculated and data were analyzed using arcsine transformation procedures.

Field Test Series 1 and 2 for FW Resistance. These tests were conducted at the Auburn University Plant Breeding Unit, Tallassee, AL, on a Wickham sandy loam (a member of the fine-loamy, mixed Thermic Typic Hapludults), which was heavily infested with both the FW fungus and RKN.

Tests were planted in a randomized, complete block design with six replications. Plots were single rows 1 m wide × 9.14 m long, with plants spaced 70 to 100 cm apart. Field FW percentages were based on both foliar and vascular symptoms early in the season and vascular symptoms only late in the season. Plants exhibiting typical symptoms of FW disease as described above were counted and removed from plots in the first week of August each year. Stems of remaining plants were cut with a longitudinal cut 40 to 60 mm long, 100 to 150 mm above the soil line, to expose vascular tissue beginning the first of October each year. Plants exhibiting typical vascular discoloration as described above were rated diseased, and both diseased and disease-free plants were counted. These counts plus diseased plant counts made in August were used to calculate disease percentages. Data on disease percentages were analyzed using arcsine transformation procedures.

The first 10 cotton cultivars and breeding lines listed above were evaluated for FW resistance in five field tests (Series 1) in 1974 thru 1978. All 18 cultivars and lines listed above were evaluated for FW resistance in three field tests (Series 2) in 1976 through 1978.

Field Test Series 3 for FW Resistance. Each cultivar and line used in this study, except for Aub 56L and Cok 310, was evaluated for FW resistance for 3 to 9 yrs in the Regional Cotton Fusarium Wilt Screening Test (Series 3) conducted at Tallassee, AL, in 1961 to 1964 by A.L. Smith (USDA-ARS) and in 1965 to 1974 by A.J. Kappelman, Jr. (USDA-ARS) cooperatively with the Department of Agronomy and Soils, Auburn University. Soil in the test field, which had the same classification as that described above, was heavily infested with both the FW fungus and RKN. Experimental procedures were similar each year with Rowden, the susceptible check, systematically grown in every 10th plot of the test. The test was planted each year in four replications with entries systematically arranged. Plots were single rows 1 m wide × 9.14 m long, with plants spaced approximately 80 mm apart in rows. Differences between FW disease percentages of each cotton and Rowden were calculated for each replication. This was done by averaging percentages of the two Rowden plots nearest a test cotton plot on each side and subtracting the percentage of the test cotton plot from this average. An overall mean difference between percentages of Rowden and that of each test cotton was then calculated

Table 1. Means of percentages of fusarium wilt disease (FW) (%) among 18 cottons in greenhouse and field tests.†

Cultivar or breeding line	Greenhouse test‡	Field test series	
		1	2
	%		
Auburn 623 RNR	30ab*	3a*	3a*
Clevewilt-6	60de	41b	25b
Auburn BR-1	59cd		25b
Bayou	63de		26b
McNair 511	29a		27b
Model	67de		26b
Coker 100A	56cd	62c	50cde
Auburn 56 Line	50bc	59c	52de
Stoneville 213	58cd	77d	67e
Deltapine 16	41b		54de
Empire WR61	57cd		56e
Coker 310	49bc		53de
Coker 201	56cd	74d	60e
Deltapine Smoothleaf	66de	95e	88f
Dixie King II	36ab	49b	36bcd
M-8	71ef	96e	94f
Delcot 277	34ab		27bc
Rowden	82f	99e	98f

* Means within a column followed by the same letter were not significantly different at the 0.05 probability level, according to Duncan's Multiple Range Test.
† Arcsines of percentages were used for the analysis of variance.
‡ Means of a series of three tests.

by using data from all replications in tests containing the test cotton.

RESULTS AND DISCUSSION

Greenhouse Tests for FW Resistance. Mean incidence of FW symptom development (hereafter referred to as greenhouse FW) ranged from 29% in McN 511 to 82% in Rowden when fungal spores were injected into the vascular systems of the 18 cotton cultivars and breeding lines in the three greenhouse tests (Table 1). McN 511, Aub 623, Del 277, and Dix K exhibited the highest resistance in the test; and Clevew, Bayou, Model, DPL SL, M-8, and Rowden exhibited highest susceptibility.

Field Tests for FW Resistance. Symptoms of FW in field tests (hereafter referred to as field FW) resulting from the RKN-FW complex occurred in all stages of growth; however, FW symptoms were usually heaviest during the fruiting period. Symptoms of FW disease ranged from slight vascular discoloration with no apparent foliar symptoms to heavy vascular discoloration with complete defoliation. Vascular symptoms late in the season were more reliable indicators of fungal infection because other factors such as heavy RKN damage and other leaf diseases frequently caused late season defoliation similiar to that caused by FW.

The 10 cotton cultivars and lines in Field Test Series 1 had from 3% mean FW in Aub 623 to 99% in Rowden (Table 1). Aub 623 exhibited the highest FW resistance in these tests. Field FW percentages were positively correlated with greenhouse FW percentages ($r = 0.74$, $P < 0.05$) in Field Test Series 1.

Eighteen cultivars and lines had mean FW disease percentages from 3% in Aub 623 to 98% in Rowden in Field Test Series 2 (Table 1). Aub 623 again had the highest resistance followed by Clevew, Aub BR-1, Bayou, McN 511, and Model. Cok 201, Stv 213, DPL SL, M-8, and Rowden were among cotton cultivars and lines with highest susceptibility in the test. The FW percentages in Field Test Series 2 also were positively correlated with greenhouse FW percentages ($r = 0.62$, $P < 0.05$).

Table 2. Mean differences between fusarium wilt disease (FW) (%) in Rowden and in each of 15 test cottons in the Regional Cotton Fusarium Wilt Screening Test, Tallassee, AL, 1961 to 1974 (Field Test Series 3).

Cultivar or breeding line†	Years tested‡	Difference§
	no.	%
Auburn 623 RNR	4	83
Clevewilt-6	4	71
McNair 511	3	66
Auburn BR-1	4	65
Bayou	4	65
Delcott 277	3	60
Model	3	58
Dixie King II	9	58
Empire WR61	7	51
Coker 100A	5	50
Deltapine 16	6	48
Coker 201	8	45
Stoneville 213	8	25
Deltapine Smoothleaf	6	13
M-8	4	6

† Arrayed in descending order of differences in FW percentages.
‡ No two cottons were necessarily tested in same year.
§ Difference = Rowden mean FW (%) minus test cotton mean FW (%).

McN 511, Model, and Del 277 were tested for 3 yrs and all others for 4 or more yrs in Field Test Series 3 (the Regional Fusarium Wilt Screening Test) (Table 2). Mean differences between test cotton cultivars and lines and Rowden in FW percentages ranged from 83% (differences between Aub 623 and Rowden) to 6% (difference between M8 and Rowden), and followed the general trends observed in Field Test Series 1 and 2 above (Table 1). Values obtained by subtracting each of these differences from 100 were correlated ($r = 0.54$, $P < 0.05$) with FW percentages obtained in the greenhouse tests.

Variation observed in greenhouse FW probably resulted from differences among cotton cultivars and lines in vascular mechanisms of resistance. Since fungal spores were injected directly into the xylem, they would have bypassed possible prevascular mechanisms of resistance that might have prevented fungal invasion of the vascular system. Such mechanisms may include microflora in the rhizosphere that are allelopathic to the fungus (5) and barriers in roots to fungal penetration of the vascular system (13). Thus, the differences observed in vascular resistance among cottons when spores of the fungus were injected into stems were most likely independent of any mechanisms of resistance that might have prevented fungal invasion of the vascular system in the field in the presence of RKN. Therefore, it appears reasonable to assume that such differences in vascular resistance were probably controlled genetically. With this assumption, McN 511, Aub 623, Del 277, and Dix K, which had 29, 30, 34, and 36% greenhouse wilting, respectively, exhibited the highest levels of vascular resistance to FW among the 18 cotton cultivars and lines (Table 1). Other researchers also have reported differences among cottons in resistance to vascular fungi after injecting fungal spores directly into the xylem (8, 11).

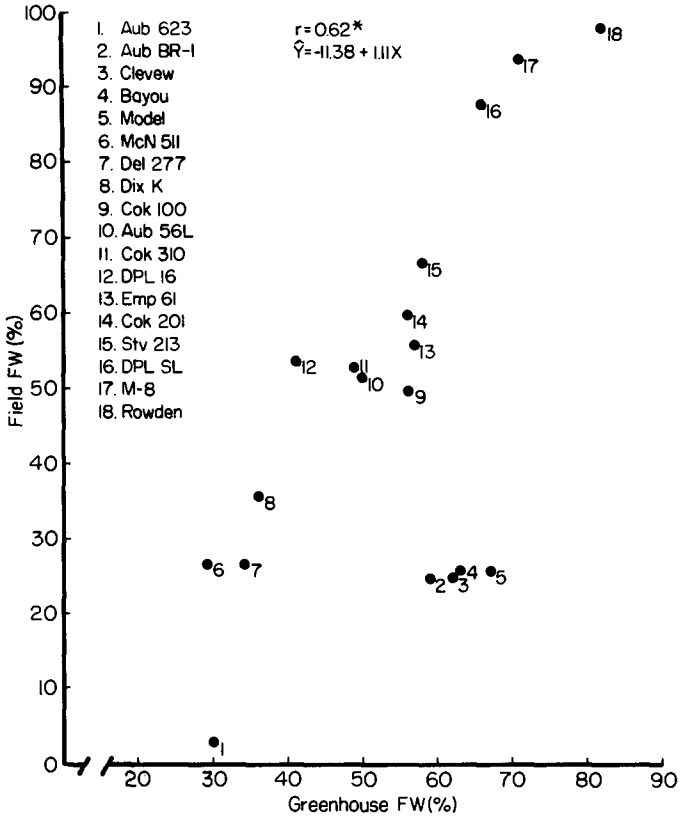

Fig. 1. Relationship of fusarium wilt disease (FW) (%) of 18 cottons grown in a field (field FW) infested with the root-knot-fusarium wilt complex to FW (%) in a greenhouse (greenhouse FW) following vascular inoculation of plants with FW spores.

Possible Mechanisms of Resistance. Resistant cotton plants, in some way, quickly recognize an invading fungus (4), or are wounded by it (9) and this induces them to initiate physical (2, 10) and/or chemical (2, 6, 13) defensive reactions against the fungus. Susceptible cotton plants do not respond in this manner or do so much more slowly than resistant cotton. Bell (4) reported evidence that recognition of an invading fungus and, a defensive reaction against it by an antigen, may be suppressed in susceptible cotton plants but not in resistant cotton plants. Beckman et al. (2) reported an occlusion process by which xylem cells of resistant cotton blocked advance of the fungus and permanently sealed it off. Fungal enzymes in susceptible cotton apparently lysed contents of xylem cells permitting systemic advance of the fungus (1, 13) and/or attached themselves to toxin receptor sites on host cell membranes causing leakage of cell solutes (9). The relationship of field wilting to RKN resistance will be presented in another paper (12).

Relationship Between Field and Greenhouse FW and its Implications on Breeding for Resistance. Squares of the correlation coefficients, reported above, between percentages of wilting in field and greenhouse tests indicate that 55, 38, and 29% of the variation in FW resistance in Field Test Series 1, 2, and 3, respectively. was accounted for by variation in FW resistance in the greenhouse tests. If we assume resistance to FW symptom development in the greenhouse indicates vascular resistance to FW, then these same percentages of variation in field FW would be accounted for by vascular resistance. Therefore, a large percentage of the variation of field FW (45 to 71%) was not accounted for by variation in vascular resistance. These results indicate that using vascular resistance to select for field resistance to FW in the presence of RKN would have limited effectiveness for breeding purposes.

The relationship between percentages of FW in Field Test Series 2 and in greenhouse tests is shown in Fig. 1. These results indicate that vascular inoculation with spores of the fungus did not differentiate between the exceptionally high field resistance of Aub 623 and the moderate field resistance of McN 511, Del 277, and Dix K. Vascular inoculation also did not differentiate between the moderate field resistance of Aub BR-1, Clevew, Bayou, and Model, and the much higher susceptibility of DPL 16, Cok 201, and Stv 213. Therefore, if breeding for field resistance to FW had been based on vascular resistance, highly resistant Aub 623 would not have been selected for having higher field resistance than that of moderately resistant McN 511, Del 277, or Dix K. Also, moderately resistant Aub BR-1, Clevew, Bayou, and Model would not have been selected for having field resistance equal to that of the latter three cotton cultivars or greater than that of highly susceptible DPL SL. Apparently Aub 623, Aub BR-1, Clevew, Bayou, and Model contain mechanisms of FW resistance in addition to vascular mechanisms that protect these cotton cultivars and lines against wilting caused by the RKN-FW complex (12). Aub 623 is highly resistant to RKN and its field resistance to FW may be due partly to its RKN resistance.

Results indicate further that the highest vascular resistance, such as in Del 277 and Dix K, that had been developed by field selection would provide only moderate field resistance to FW in the presence of RKN. The level of vascular resistance in Aub 623, McN 511, Del 277, and Dix K may be the highest available in cotton. Cotton cultivars probably can be selected for moderate resistance to FW in the RKN-FW complex based on vascular inoculation with FW spores. However, the probability of developing cotton cultivars with high resistance using this method appears to be low.

Cotton selected in the field for resistance to FW in the RKN-FW complex may contain one or a combination of the mechanisms of resistance discussed above. There apparently would be a low probability of developing upland cotton cultivars highly resistant to FW by field selection. This is supported by results of this study and by the lack of reported evidence that high FW resistance has ever been developed in upland cotton through field selection even though this was the primary method of selecting for FW resistance since the beginning of this century. Failure in the past to develop cotton cultivars with high FW resistance by field selection may have been due partly to the inability to apply intensive selection pressure for FW resistance and partly to the absence of genes for high FW resistance. Natural populations of the FW fungus and the nematodes that enable it to invade and develop in plants are highly variable. This contributes to the difficulty of applying intensive selection pressure

in the field.

While no upland cotton is known to contain high FW resistance resulting from field selection, resistance has been improved gradually over the years by this method (Fig. 1). Cotton cultivars and lines in Fig. 1 can be placed in resistant, moderately susceptible, susceptible, and highly susceptible groups according to their levels of FW resistance in the field. The highly susceptible group, exemplified by Rowden, was developed earliest and probably contained resistance representative of cultivars in the early 1900s when intensive breeding for FW resistance began. The susceptible group, which averaged approximately 55% wilted plants and exemplified by Stv 213 and DPL 16, represented approximately 40% improvement in FW resistance compared with that of the highly susceptible group. Many of the cotton cultivars and lines in the susceptible group had been developed 10 to 20 yrs prior to 1965. The moderately susceptible group, which average approximately 30% wilted plants and exemplified by Del 277, represented approximately 65% improvement in FW resistance compared with that of the highly susceptible group. Most of the cotton plants in the moderately susceptible group originated more recently than those in the susceptible group, and they represent the highest FW resistance developed in cotton through field selection. Auburn 623, the only cotton in the resistant group, had been developed through selection for RKN resistance. Utilizing genes for higher FW resistance, such as in Aub 623 and possibly in primitive cotton germplasms, and developing more precise screening techniques possibly will allow successful future field selection in developing agronomically elite cultivars with higher resistance than is presently available.

Results of this study indicate that additional research is needed to identify all factors that provide field resistance to FW disease. A better understanding of such factors would help in identifying selection criteria and in developing more rapid and precise techniques for screening for resistance than are now available.

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