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Brief Articles

GREENHOUSE EVALUATION OF VERTICILLIUM, FUSARIUM, AND ROOT KNOT NEMATODE ON COTTON[1]

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ABSTRACT

Cotton (*Gossypium hirsutum* L.) plants 3 or 6 weeks old were inoculated with *Fusarium oxysporum*, f. sp. *vasinfectum, Verticillium albo-atrum*, or *Meloidogyne incognita acrita*, individually and in all combinations, to determine an inoculation procedure that would permit an evaluation for multiple disease resistance. Four cotton varieties of known disease reaction were used for comparison. Differences in varietal response to *F. oxysporum* were best expressed in the younger plants, and varietal differences to *V. albo-atrum* were best expressed in older plants. Although varietal differences in wilt response were less clear when both fungi were inoculated simultaneously, these differences were more evident in the younger plants.

Most stunting occurred when plants growing in root-knot nematode-infested soil were inoculated with either or both pathogens. Good differentiation of varietal resistance was not apparent. Only the most susceptible and most resistant varieties could be distinguished when infected with all 3 pathogens.

Additional index words: Gossypium hirsutum L., *G. barbadense* L., Multiple-resistance.

THREE important soil-borne pathogens of upland cotton (*Gossypium hirsutum* L.) are *Verticillium albo-atrum* Reinke & Berth., (microsclerotial form) *Fusarium oxysporum* f. sp. *vasinfectum* (Atk.) Snyd. & Hans., and the root-knot nematode *Meloidogyne incognita acrita* (Chitwood) Chitwood and Oteifa. *F. oxysporum* and *M. incognita* are most prevalent in light-textured soils. Both pathogens commonly attack cotton simultaneously and cause more damage than if either pathogen attacks alone (3). Minton, et al. (5) have shown in the greenhouse that a response to *F. oxysporum* was most severe in the presence of root-knot or sting nematodes but was not affected by soil type. Symptoms of this complex are stunting and chlorotic and finally necrotic leaves. Leaves often remain on the plant. Death of the entire plant results under severe situations.

V. albo-atrum is prevalent in heavy-textured and loamy soils. Usually it is not associated with *M. incognita*. In one case where the two were associated, fumigation reduced symptom expression of root-knot but not *V. albo-atrum*, indicating that the root-knot nematode was not necessary for severe verticillium wilt as is the case with fusarium wilt (4). *V. albo-atrum* invades the cotton plant systemically, causing defoliation and stunting. Death may occur if young plants are attacked.

In many sections of the Mississippi Delta, cotton is grown on both light- and heavy-textured soils. Mixed soil types exist in transition zones where all three pathogens may infect cotton (personal observation). Under these situations a successful cotton variety must be resistant to all three pathogens if it is to be grown over a large area. This prompted studies to develop an inoculation procedure that would permit an evaluation of resistance to all three pathogens in the greenhouse. Differences in varietal response to *F. oxysporum* and *V. albo-atrum* in the greenhouse have been distinguished with stem inoculations (1, 2). These responses correspond with known varietal differences. The addition of *M. incognita acrita* to the disease complex required new investigations.

MATERIALS AND METHODS

Three varieties of *Gossypium hirsutum* L. 'Stardel,' 'Delcot 277,' and 'MO-DEL' and one variety of *G. barbadense* L., 'Sea Island Seabrook' (SISB), were used for comparison. Stardel is susceptible to all three pathogens used here, and SISB is resistant to *Fusarium* and *Verticillium* but susceptible to the root-knot nematode. MO-DEL and Delcot 277 are varieties released by the Missouri Agricultural Experiment Station, 1968, 1970, respectively. MO-DEL was selected for resistance to the *Fusarium* root-knot nematode complex and Delcot 277 for resistance to *V. albo-atrum*. Delcot 277 is susceptible to the root-knot nematode. Cotton was planted, one plant per 23-cm pot, in steamed or nematode-infested soil at two time intervals to give plants 3 and 6 weeks old at inoculation. Each treatment was applied to one plant of each variety and was replicated four times in a complete randomized block design.

Nematode inoculum. The following method was used to increase *M. incognita acrita* and at the same time help reduce

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the numbers of other plant parasitic nematodes: (i) tomato plants were grown in soil infested with the root-knot nematode; (ii) roots bearing galls were washed, chopped, and mixed with steam-treated soil; (iii) cotton seed were planted in this mixture, and galls containing larvae and egg masses developed on the stems 1 to 5 cm above the soil line; (iv) cotton stems with galls were cut and placed with cotton seed in steamed soil; the roots of these plants developed typical root-knot symptoms 5 weeks later; (v) the aboveground portions of seedlings were discarded, and the roots and soil were mixed and diluted with steamed soil. Nematodes were extracted from 50-ml samples of this soil, using the Baermann funnel method. There were 2 to 4 nematodes/ml of soil at the beginning of the test.

Fungus inoculum. The isolates of *F. oxysporum* and *V. albo-atrum* were collected from cotton in southeast Missouri. The inoculum for each organism was increased in a Czapek's broth shake culture for 3 to 4 days. The culture was filtered through cheesecloth to remove mycelia. Most of the spores produced by *F. oxysporum* were micro-conidia. Concentrations of conidial suspensions were determined with a hemocytometer and adjusted to 3×10^6 conidia/ml. Plants were inoculated with conidia by stem injection (2). Inoculum was delivered from the syringe to form a bead of suspended conidia at the tip of the needle. The needle was injected into the stem until the bevel point was just visible. The drop of inoculum that formed in the axis of the stem and needle disappeared into the stem, giving visual evidence of inoculation. In one test with *V. albo-atrum*, beads of conidial suspension were diluted and aliquots were plated in molten agar. Colony counts showed that beads of a 3×10^6 conidia/ml suspension contained a maximum of 60,000 conidia. This should also be true for micro-conidia of *F. oxysporum* because they were adjusted to the same concentrations as *V. albo-atrum*. Injections were made at three sites equidistant around the circumference of each stem, about 2 cm above the soil. Check plants were injected with sterile distilled water. The greenhouse temperature range was 26 C to 30 C.

Plants were severed at the soil line 4 weeks after inoculation. They were dried at approximately 38 C for 48 hours and then weighed.

RESULTS AND DISCUSSION

Symptoms of verticillium wilt began 4 days after inoculation. Epinasty of terminal petioles was followed by wilting and chlorosis. Defoliation occurred on susceptible plants. Symptoms of fusarium wilt began 5 days after inoculation. Vein darkening was followed by wilting, chlorosis, and necrosis of leaves. The leaves remained attached to the plant. Symptoms appeared to progress at the same rate when the inoculum of *F. oxysporum* and *V. albo-atrum* were combined.

Effect of nematodes alone. Stunting was evident on only those plants that had grown in nematode-infested soil for 10 weeks. But the differences between the two ages or among the varieties were not statistically significant. Delcot 277 was the only variety that showed a significant weight loss after 10 weeks when compared to the 7-week-old plants (Table 1).

Effect of fungi alone. Differences in the varietal response to infection by *F. oxysporum* or *V. albo-atrum* depended on the age of the plants and whether nematodes were present. In the absence of nematodes varietal differences of resistance to *V. albo-atrum* were expressed more clearly in older plants than in younger plants. Plants of SISB, infected when 6 weeks old, were more resistant than the other three varieties. But if infected when 3 weeks old, all varieties appeared equally susceptible. When plants were infected with *F. oxysporum* the 3-week-old plants showed greater varietal differences than 6-week-old plants. Here, Stardel was least resistant. All varieties, even the most susceptible Stardel, expressed a resistant reaction to *F. oxysporum* if infected when 6 weeks old.

Table 1. Dry weight of inoculated plants expressed as a percentage of controls.

Plants inoculated with			Dry weight (% control - *)							
			Stardel		SISB		Delcot 277		MO-DEL	
Fusarium	Verticillium	Meloidogyne	3 wks	6 wks	3 wks	6 wks	3 wks	6 wks	3 wks	6 wks
+			48l-r	90a-d	82b-h	100a-b	78c-i	88a-d	75d-j	85a-f
	+		30r-t	46l-s	47l-s	87a-e	44m-s	64f-m	42o-s	56j-p
		+	106a	85a-f	100a-b	84b-g	103a-b	74d-k	98a-c	92a-d
+	+		33q-t	49l-r	63g-n	66e-l	58l-p	63g-n	44m-s	62h-o
+		+	34q-t	55j-p	43n-s	54k-p	50l-q	57j-p	46l-s	60i-p
	+	+	28s-t	22t	38p-t	40p-t	40p-t	40p-t	42o-s	38p-t
+	+	+	33q-t	34q-t	60i-p	47l-s	54k-p	55j-p	44m-s	43n-s

*Control plants were grown in steamed soil and injected with sterile distilled water. Means with same letter are not significantly different based on Duncan's test at 5% of probability.

When older plants were inoculated with both fungi simultaneously, the dry weights of all varieties did not differ significantly from those inoculated only with *V. albo-atrum*. The dry weight of SISB was similar to that of Delcot 277 and MO-DEL. But with the younger plants, SISB and Delcot 277 were more resistant than Stardel. Simultaneous inoculation with both fungi did not result in the clear expression of varietal resistance that occurred when each fungus was inoculated individually.

Effect of nematodes and fungi. The dry weights of plants that were inoculated with the fungal pathogens while growing in root-knot-infested soil were expressed as percentage dry weights of control plants grown in steamed soil and injected with sterile distilled water. Plants that were infected with the nematode plus either fungus were stunted more than those infected by either fungus alone. This was true even for the *V. albo-atrum* and nematode combination, a point not well recognized because these two pathogens do not usually survive well in the same soil environment.

Stardel was more resistant at 6 weeks than at 3 weeks when inoculated with the *Fusarium*-nematode combination. This resistance was not evident when *Verticillium* was included. Differences among varieties was apparent only between Stardel and SISB. SISB was more resistant than Stardel when all three pathogens infected plants at 3 weeks of age. There were no significant differences among other varieties, inoculum treatments or between ages at inoculation.

These results offer some guidance for a program designed to develop cotton varieties that are resistant to *V. albo-atrum, F. oxysporum* and *M. incognita*. The data indicate that populations of unknown disease resistance should be tested against each pathogen separately. This would permit the identification of the highest forms of resistance. The best expression of resistance to *F. oxysporum* occurred when plants were inoculated when 3 weeks old and with *V. albo-atrum* when the plants were 6 weeks old. When *V. albo-atrum* was inoculated alone, the variety SISB was clearly most resistant, as it is under field conditions. When *V. albo-atrum* was combined with *F. oxysporum* or *M. incognita*, or if all three were combined, this resistance was obscured.

When plants were attacked by all three pathogens, only SISB expressed resistance significantly greater than Stardel. It is suggested that the 3-way inoculation may be of use in assessing advanced progeny. Previous field experience has indicated that high levels of resistance to the *F. oxysporum-M. incognita* complex also is associated with tolerance to *V. albo-atrum* (6). Selections in this direction can now be initiated in the greenhouse.

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AN EXAMPLE OF INCREASED RECOMBINATION IN *GOSSYPIUM*[1]

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ABSTRACT

A chromosome bearing a marked segment was transferred from the diploid species *Gossypium raimondii* Ulb. to the teraploid species, *Gossypium hirsutum* L. Upon mating with the homeologous *G. hirsutum* chromosome, the segment gave 2.4% recombination for the two markers used. When the genes were later recombined from the repulsion phase, the recombination value increased to an average of 20.2%. This increase was attributed to accelerated recombination in homologously paired segments that appeared in the intercalary region following initial breakage.

***Additional index words:* Gossypium hirsutum L., Gossypium raimondii Ulb., Interspecific hybrid.**

INTERSPECIFIC transfer of chromosomal material frequently results in reduced recombination in a given segment when the introduced chromosome pairs with a "native" strand. Moreover, there is often an increase in chiasmata number at adjacent sites so that the frequency of chiasmata for the bivalent in question might not be reduced below that of the control species (10). Using interspecific hybrids in cotton (*Gossypium* spp.) Rhyne (8) showed that there were increases in recombination in homospecifically paired chromosomal regions that were adjacent to heterospecifically paired segments. There were accompanying decreases in recombination in the latter regions. Thus it appears that shifts in chiasmata placement are accompanied by changes in recombination frequency when "foreign" germplasm is introduced into what had been, previously, a stable species. The present report concerns a striking example of what appears to be such a displacement of recombination following the introduction of an alien chromosomal segment into cultivated upland cotton.

PROCEDURES, RESULTS, AND DISCUSSION

A chromosome, or a segment thereof, bearing the linked genes, Gl_3^{rai} and Bw_2^{rai}, was transferred from the American diploid species, *Gossypium raimondii* Ulb., to tetraploid upland cotton, *Gossypium hirsutum* L. The gene Gl_3^{rai} determines normally glandular plant, and Bw_2^{rai} normal bractiole. The recessive alleles of these genes condition glandless plant and withering bractiole. The *G. hirsutum* recipient stock was homozygous for both recessive alleles. Henceforth, for the sake of brevity, the dominant alleles will be designated *Gl* and *Bw*, and the recessive alleles *gl* and *bw* respectively. These genes are in the D sub-genome of *Gossypium* (7)

A tetraploid plant of the genotype Gl-Bw/gl-bw was crossed with the homozygous recessive stock and the BC_1 progeny scanned for evidence of recombination. The results are given in Table 1. The average recombination value of 2.4% is strikingly lower than the 12 to 17% calculated by Rhyne (9) for the homeologous linkage group in the A sub-genome of *G. hirsutum*.

The recombinant individuals were saved and intermated. From their progeny plants were selected that held *Gl* and *Bw* in repulsion. Several of these were planted in the field and in the greenhouse and crossed with the recessive tester stock. The progenies of the individual plants were scored separately, and the data are presented in Table 2. Homogeniety estimates for linkage were calculated according to Fisher's scoring method (4) as revised by Kramer and Burnham (6). Since progenies from greenhouse and

Table 1. Linkage estimates for coupling data.

Population number	Class frequencies				Recombination value
	gl bw	gl Bw	Gl bw	Gl Bw	
1	241	3	2	236	.010
2	91	9	3	102	.058
3	70	2	1	62	.022
Total	402	14	6	400	.024 ± .0038*

* Homogeniety chi-square for linkage = 7.02, 2d.f.; sig. at .05.

Table 2. Linkage estimates for repulsion data.

Plant number	Class				Recombination value
	gl Bw	gl bw	Gl Bw	Gl bw	
1	66	22	19	61	.244
2	65	15	9	49	.174
3	130	19	32	127	.166
4	89	20	25	83	.203
5	93	22	35	77	.251
6	34	12	8	21	.267
7	105	20	40	83	.242
8	55	11	11	51	.172
9	85	21	18	70	.206
10	128	22	39	106	.207
11	157	35	48	124	.228
12	70	13	13	81	.147
13	44	3	15	27	.202
14	35	7	9	28	.202
15	48	12	20	47	.252
16	31	5	14	28	.244
17	41	8	12	28	.225
Total	1276	267	367	1091	.202 ± .0047*

* Homogeniety chi-square for linkage = 11.13, 16 d.f.; N.S

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