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Effect of Verticillium Wilt on Cotton Yield, Fiber Properties, and Seed Quality[1]

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ABSTRACT

Three varieties of upland cotton (*Gossypium hirsutum* L.) that differed in susceptibility to *Verticillium albo-atrum* (microsclerotial form) were planted on three dates at 10-day intervals. They were inoculated with *V. albo-atrum* by stem puncture in July, August, and September. Most of the damage to fiber properties and seed germination resulted from the August inoculation when bolls were in early stages of maturation. Most of the seed weight loss occurred following the September inoculation. No reduction in yield, fiber properties, or seed weight and only occasional loss of germination occurred following the July inoculation.

Poor quality seed from infected plants resulted in poor stands. The amount of damage correlated with known varietal susceptibility. This method of inoculation permitted detailed assessment of resistance to *V. albo-atrum* under field conditions.

***Additional index words:** Verticillium albo-atrum, Gossypium hirsutum* L.

VERTICILLIUM wilt is one of the most destructive diseases of cotton (*Gossypium hirsutum* L.) in the U.S. Not only does the disease reduce the yield of lint, but the fiber produced on diseased plants is of inferior quality. Length, strength, and fiber fineness are adversely affected, and grades are lowered. When plants are infected during early stages of boll development, the seed produced have low germination ability. The resulting seedlings lack vigor and are more susceptible to seedling diseases (2, 8). *Verticillium*-infected seedlings are usually stunted and many of them may die. The stage of plant development when infection occurs has considerable influence on the amount of damage that will result. Late season infections cause little reduction in yield and practically no damage to fiber or seed.

There are few reports of precise measurements of the damage to seed and fiber caused by verticillium wilt. One report (5) compared the damage caused by *Verticillium albo-atrum* to one susceptible and two resistant varieties of cotton. Germination dropped 10% in the seed from the susceptible variety but only 5% in seed from the resistant varieties, and fiber quality was seriously affected in severely infected susceptible plants (5). Another report (7) showed the amount of damage to fibers taken from diseased plants. There is no information on how much damage might occur when plants are infected at certain stages of development. The inoculation technique we used allowed us to gain this information.

MATERIALS AND METHODS

The experimental design was split-split plot. Planting dates were whole plots; dates of inoculation were subplots; varieties were sub-subplots; and inoculated or uninoculated checks were sub-sub-subplots. Treatments were replicated four times and randomized in a complete block design for each date of planting. Two year's data were combined and analyzed by analysis of variance. Treatment means were compared by Duncan's new multiple range test or the least significant difference test at 5%.

Gossypium hirsutum L., 'Stardel' was the susceptible host; 'Delcot 277' was the resistant host. The latter was bred in Missouri for resistance to *Verticillium albo-atrum* Reinke and Berth. 'Deltapine 45A' was used because its wilt reaction was intermediate between the other two varieties and it is recommended in certain wilt areas. The varieties were planted in four-row plots, 7.6 m long on May 10, 20, and 30, 1967 and May 21, 31, and June 10, 1968. Planting dates of May 1 to 15 were optimum for maximum yield in southeast Missouri. Hills were 0.3 m apart and the stand was thinned to one plant per hill. The plants were inoculated by using a needle and syringe to inject conidia ($3\text{-}4 \times 10^6$/ml) into the stems 3 to 6 cm above the soil line (4). Inoculations were made in (*i*) mid-July 4 to 15 days before flowering, (*ii*) mid-August at early boll development and (*iii*) mid-September at or near boll dehiscence. Plants that showed wilt symptoms in check plots or in treated plots before inoculation were tagged and not sampled. An isolate of *V. albo-atrum* that was collected from cotton in southeast Missouri was used. Inoculum was produced in Czapek's broth as a shake culture for 4 days at 25C.

Samples of 100 bolls were hand-picked from entire plants in the two middle rows. Random samples of 150 seeds from each replicate were rolled in paper towels, placed in glass beakers with enough water to saturate the towel, and placed at constant 18, 24, or 29C for germination. Final germination counts were made 7 days later. The Cotton Quality Spinning Laboratory of the U. S. Department of Agriculture, Knoxville, Tenn. analyzed lint samples for fiber characteristics.

RESULTS

Wilt symptoms began about 12 days after inoculation. The severity of wilt among the three varieties

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correlated with known resistance. In 1967, the number of stem inoculated plants that appeared symptomless decreased after each inoculation date. After the first inoculation date, 21% of 2,729 inoculated plants escaped; second date 7% of 2,827 escaped; third date 0.6% of 2,892 escaped. This trend reflects the higher resistance of young cotton early in the season. Fewer escapes occurred when plants were inoculated after boll development began. In August of 1969, only 0.7% of 1,328 stem inoculated plants in border rows of a wilt nursery escaped infection.

Yield. In 1967, the season was cool and short, so average maximum yields were not attained. In 1968, an early frost permitted the collection of only 100-boll samples; total yield was not taken. In 1967, healthy plants of all varieties yielded the same. Stardel was the only variety in which infected plants yielded significantly less than the healthy checks. Delcot 277 produced considerably more lint than Stardel but yielded more than Deltapine 45A in only one instance under wilt conditions; i.e., the August inoculation on the second planting date. The first and second planting of infected Delcot 277 yielded more than the healthy checks. This unexpected result was due to the short season which prevented bolls on healthy plants from opening fully; but sufficient defoliation of infected plants permitted aeration followed by drying and opening of bolls.

Boll size. The yield of seed cotton per boll was not reduced when plants were infected in July. When infected in August, all varieties suffered a loss of seed cotton per boll. When plants were infected in September, seed cotton per boll was reduced in (*i*) Stardel of all ages, (*ii*) youngest plants of Delcot 277 and, (*iii*) intermediate aged plants of Deltapine 45A. Infected plants of Delcot 277 produced considerably more seed cotton per boll than the other two varieties.

Span length, 2.5%.—Length which is equalled or exceeded in inches by 2.5% of the fibers. The length of fibers was significantly reduced by verticillium wilt only in the oldest plants of all varieties that were inoculated in August. The fibers of Delcot 277 were about 0.1 inch longer than those of the other two varieties, whether infected or not. The fiber lengths of Deltapine 45A and Stardel were comparable.

Yarn strength. Yarn strength was significantly reduced only when infection occurred in August on plants of the second planting date. Delcot 277 had the strongest yarn compared to the other varieties whether inoculated or not. Stardel produced stronger yarn than Deltapine 45A, whether infected or not.

Micronaire.—The fineness of the fiber. Satisfactory fineness of cotton lint is 3.5 to 4.8 micronaire units. The cool, short season during 1967 caused extremely fine fiber (1.5 to 3.0 micronaire units); fineness in 1968 was normal. Therefore, the micronaire averages in Table 1 are below (too fine) desired quality standards. But the effects of wilt can still be detected. Healthy plants of Delcot 277 produced fibers that were finer than those of the other varieties, except in late plantings when all varieties were comparable. This agrees with previous variety tests. Delcot 277 (oldest plants only) had lower micronaire than the other varieties after the August inoculation dates. Stardel had low micronaire under the same circumstance. Deltapine 45A had low micronaire only when the youngest plants were inoculated in September.

Seed Weight. Plant age did not significantly affect seed weight of infected Stardel or Deltapine 45A. Delcot 277 seeds weighed least when the youngest plants were inoculated in September (Table 1). Comparisons with appropriate uninoculated check plants show that the most reduction of seed weight due to verticillium wilt occurred when plants were infected in September. Seed from infected Stardel plants of all ages weighed less than those from the checks, when inoculated in September; but when they were inoculated in August, only seeds from plants of the second planting date were lower than those of the checks. Seed weights for infected Deltapine 45A were lower than those for the checks in plants from the first and second planting date when inoculated in September. Only seed weights from the youngest infected plants of Delcot 277 were lower than those of the checks when inoculated in September. Reductions in seed weight ranged from 10% (Delcot 277 and Deltapine 45A) to 14% (Stardel) calculated as: check — treated/check.

Germination. Percentage of germination of seed produced by infected plants was less than that of seed produced by healthy plants. This reduction was sig-

Table 1. The effect of *Verticillium albo-atrum* on the fiber and seed quality of three varieties of *Gossypium hirsutum* when planted and inoculated on three different dates. (Combined analysis of data from 1967 to 1968).

Date of planting†	Date of inoculation	Lint‡ yield	Boll size	2.5% span length	Yarn strength	Micronaire	Seed index	Germination
		kg/ha	g, SC	in.	22's	units	g	%
				Delcot 277				
1	July	655	7.45	1.25	147	2.1	13.6	84
2		716	7.20	1.24	142	2.3	14.2	80*
3		369	6.71	1.23	144	2.1	13.8	75
1	August	631	6.63*	1.20*	140	1.6*	12.3*	82
2		693	6.31*	1.20	139*	2.1	12.8	81*
3		393	6.36	1.21	138*	2.1	12.7	54*
1	Sept	800	6.86	1.24	147	2.0	12.7	86
2		870	7.11	1.23	144	2.1	13.1	83
3		415	5.56*	1.20	144	1.2*	11.3*	76
				Deltapine 45A				
1	July	546	5.98	1.14	132	2.5	11.4	83
2		593	6.08	1.14	129	2.6	12.2	81
3		292	5.65	1.17	134	2.3	12.1	64*
1	August	516	5.49*	1.10*	130	2.3	11.1	75*
2		524	5.65	1.11	126*	2.5	11.5	71*
3		292	5.68	1.16	131	2.2	11.6	55*
1	Sept	662	5.59	1.14	131	2.4	10.4*	84
2		631	5.25*	1.11	132	2.4	10.8*	83
3		316	4.86	1.13	132	1.6*	10.0	68
				Stardel				
1	July	369	5.91	1.17	139	2.5	12.1	72*
2		369*	6.06	1.13	138	2.5	11.9	78
3		185	5.54	1.15	140	2.4	12.0	60
1	August	431	5.03*	1.12*	136*	2.1*	10.6	75*
2		393	4.88	1.09*	131*	2.3	10.1*	73
3		177*	4.88	1.14	137	2.1	10.8	58*
1	Sept	631	5.05*	1.14	143	2.3	10.3*	85
2		532	4.65*	1.13	139	2.1*	10.3*	79
3		346	4.45*	1.16	141	1.8*	9.6*	68
				Delcot 277				
1	Checks	608	7.28	1.25	145	2.0	13.3	88
2		669	6.95	1.22	145	2.2	13.2	90
3		270	6.28	1.23	146	2.0	12.5	74
				Deltapine 45A				
1		439	6.11	1.15	131	2.6	11.6	86
2		616	5.93	1.14	132	2.6	11.7	80
3		292	4.78	1.15	136	2.0	10.1	75
				Stardel				
1		532	5.71	1.16	143	2.5	11.4	86
2		570	5.34	1.14	138	2.5	11.2	75
3		362	5.33	1.14	139	2.3	11.2	68
LSD, 5%		181	0.54	0.03	5	0.3	0.8	8

* Means followed by an asterisk are significantly lower than uninoculated checks of the same variety and date of planting based on Duncan's new multiple range test, 5%.
† 1967; 1 = 5/10, 2 = 5/20, 3 = 5/29; 1968; 1 = 5/21, 2 = 5/31, 3 = 6/10. ‡ Lint yield is for 1967 only.

nificant if infection occurred in August or occasionally in July, but never in September. This relationship existed for each germination temperature so only the results of 24C are given in Table 1. Seed from infected Deltapine 45A of all ages had reduced germination compared to uninoculated controls following the August inoculation. Seed from infected Delcot 277, planted on the second and third dates, had reduced germination compared to controls following the August inoculation. Stardel seed from the oldest plants inoculated in July and August had a lower germination percentage than controls. An analysis of data—combining varieties, years, and planting dates—showed that seeds produced after the July and August inoculation had significantly lower germination ability than those from the healthy plants, or those from plants that were inoculated in September. The September inoculation had no apparent affect on germination.

Stand counts. Samples of seed produced by Stardel and Delcot 277 were planted in one-row plots in the field in 1969. The seeding rate was adjusted to produce 121,000 plants/ha. Seed samples came from the August inoculation of the first (normal) and third (late) planting dates. The seed were acid delinted but not screened or treated with a fungicide. The treatments were replicated eight times in a completely randomized block design. Stand counts were taken 30 days later. The data in Table 2 show that age at infection, variety, and year influenced the stands of cotton. Seed from the first year, from both inoculated and healthy plants produced a lower stand than seed from the second year. Lowest stands resulted when seed came from the youngest plants that were inoculated. Seed from infected Delcot 277 produced higher stand counts than those from infected Stardel. This was most obvious in the 1967 seed.

Table 2. Stand counts from seed produced by normal or late cotton infected with *Verticillium albo-atrum*. A perfect stand based on 90% germination: 121,079 plants/ha.

Variety	Planting date	Plants/ha of following crop (1,000)*	
		Inoculated	Check
	1967 seed		
Stardel	normal	70 i	109 c
	late	31 k	107 c
Delcot 277	normal	93 f	99 e
	late	53 j	93 f
	1968 seed		
Stardel	normal	95 f	117 b
	late	87 g	78 h
Delcot 277	normal	102 d	121 a
	late	87 g	93 f

* Means followed by the same letter are not significantly different (DNMRT, 5%)

DISCUSSION

Damage to fiber properties or seed weight did not result from the July inoculation (4 to 15 days before flowering). Cotton was reported to be less susceptible to *V. albo-atrum* in the field before flowering (9). Wilt symptoms were observed following the July inoculation, but recovery permitted the production of a normal crop. Infection was assured because of the inoculation technique (4). *V. albo-atrum* must have been permanently retarded at the inoculation site, otherwise pathogenesis would have resumed upon flowering, and it would have affected fiber and seed properties.

It takes about 40 to 80 days in Missouri for a cotton boll to mature from the open flower to dehiscence. This is influenced largely by temperature. Maximum seed size and fiber length is attained during the first third of the boll maturation period. Maximum seed weight and fiber strength and weight are not attained until boll maturation is nearly complete (10). This explains why the most severe damage occurred following the August inoculation when bolls were in early stages of maturation. The reduction in the rate of maturation caused by wilt did not allow full development of fiber and seed properties by harvest time. Damage after late season inoculation is reflected in the weight of seed cotton per boll, seed weight, and micronaire especially in the late maturing, susceptible Stardel. These are characters which are not fully developed until late in boll maturation.

Brown et al. (3) found no reduction in seed index or germination if bolls were 35 days old or more when chemically defoliated. Our results show a loss in seed index in certain instances after the September inoculation when bolls were more than 35 days old.

Evidently the factors responsible for optimum percentage of germination were fully developed by September, because percentage of germination was not reduced following infection at this time. This suggests that *V. albo-atrum* can cause a reduction in seed weight but not necessarily percentage of germination after late season infection. Germination was reduced as much as 20% in late planted cotton when infection occurred early to mid-way through boll maturation. Seed weight was reduced 10 to 14% when infection occurred in late stages of boll maturation. But a satisfactory germination percentage may not reflect low vigor in light seed.

Environmental factors in the field in 1969 were not favorable for the development of seedling disease. Seed were relatively free of pathogens because they were harvested by hand and delinted with sulfuric acid. Therefore, the stand counts in Table 2 mainly reflect vigor and germination ability under field conditions. The high temperatures during 1968 were not conducive to maximum verticillium wilt develop ment. This accounted for the high quality seed and satisfactory stand counts in the seedling disease test. In southeast Missouri a satisfactory stand count is 86,000 to 148,000 plants/ha. The drastic effect on the quality of planting seed was apparent when 29 to 69,000 plants/ha were produced by seed taken from wilted plants. The use of this seed would require a seeding rate about triple that of normal in order to obtain a satisfactory stand count.

The 1967 season was cool and short. Bolls did not open fully on healthy, nondefoliated plants. Partial to complete defoliation occurred after the September inoculation, which allowed bolls to dry and open sooner in the infected than in the healthy plants. Because of this, the average yield for the entire test was 95 kg/ha greater from the inoculated than from the healthy plants. A reduction in micronaire and seed weight was the only appreciable damage. This accounts for the significant yield increase of infected over healthy Delcot 277 and suggests that late season defoliation, even if biologically induced, may benefit yield and not injure other components of the crop.

Reliable and reproducible results can be obtained with the stem puncture method of inoculation (4, 6). Recently the method was used to study the inheritance of tolerance to *Verticillium* in cotton (1). Our results indicate that the method can be used on a large scale in the field to measure the resistance to *Verticillium* that is expressed in various components of the cotton crop. When infected with *Verticillium,* Delcot 277 was superior to Deltapine 45A and Stardel with respect to seed cotton per boll, fiber length and strength, and stand counts from seeds produced by infected plants.

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