

## Heterozygosity in Inheritance of *Verticillium* Wilt Tolerance in Cotton

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### ABSTRACT

F<sub>1</sub> responses of two cotton strains (*Gossypium hirsutum*) Acala 9519 and Acala 1479, tolerant to *Verticillium* wilt, when crossed with Acala 227, a susceptible strain, differed from each other and with respect to the individual tolerant parent plant used. This varied response was attributed to heterozygosity of the tolerant parent plants. The require-

ments for starting genetic analysis of *Verticillium* wilt tolerance are discussed. Tolerance to the mild SS-4 isolate of *Verticillium albo-atrum* acted as a dominant character(s) in F<sub>1</sub> plants of the A9519 × A227 and A1479 × A227 crosses. *Phytopathology* 60:301-303.

The inheritance of tolerance to *Verticillium albo-atrum* Reinke & Berth. in cotton is not well understood. Some tolerant cotton strains do not transmit their tolerance, while others produce tolerant progenies regardless of the other parent (3). Some crosses demonstrate transgressive segregation under field conditions (3, 4).

Samayoa et al. (5) found that colonization of cotton plants with *V. albo-atrum* was retarded under a diurnal cycle of a 36.5 C temperature day and an 18.0 C temperature night. Symptom expression was either mild or absent. Plants grown under this regime for 45 days and transferred to a 29.0 × 19.0 C regime expressed differences between susceptible, tolerant, and resistant cotton strains. They also noted more severe symptoms under a regime of 26.5 C day and 18.0 C night than at constant 22.0 C or under a 31.0 C × 18.0 C regime with the same light.

Bell & Presley (1) observed that susceptible, tolerant, and resistant varieties of *Gossypium* sp. were susceptible when inoculated with a defoliating isolate of *V. albo-atrum* at 22 C. Symptom expression decreased as temperature increased in all varieties to 32 C, where all varieties expressed a resistant reaction.

Tolerant and susceptible expressions were uniform and repeatable in tolerant Acala 9519 and susceptible Acala 227 cotton strains when inoculum, plant size, and temperature were rigidly controlled. (J. R. Barrow, unpublished data). The purposes of this study were to determine the degree of homozygosity in parental cotton plants and to begin an inheritance study by rating F<sub>1</sub> plants from crosses between susceptible and tolerant plants.

**MATERIALS AND METHODS.**—Acala 9519 and Acala 1479 were obtained from New Mexico breeding stocks. Both strains show field tolerance to *V. albo-atrum*. The selection of A9519 is from 2507 × Hartsville 49W × 49 × 1517-C. A1479 is a selection from Hopicala × 2503 × Coquette. Seed from highly susceptible strain Acala 227 were provided by W. D. Fisher, Cotton Research Center, Phoenix, Arizona. Cultures of defoliating and nondefoliating isolates (T-1 and SS-4, respec-

tively) of *V. albo-atrum* were provided by W. C. Schnathorst, Davis, California.

Two experiments were conducted with F<sub>1</sub> plants of the A9519 × A227 crosses and one experiment with F<sub>1</sub> plants of the A1479 × A227 crosses. Fruits were individually harvested and processed; the seeds were acid-delinted and hot-water treated (80 C for 3 min). Prior to planting, seeds were treated using a procedure modified from that of R. M. Taylor, Far West Research Station, El Paso, Texas, (*personal communication*) to increase the rate and percentage of germination. A section of seed coat was removed from the side of each seed. The seeds were then covered with filter paper (saturated with sterile distilled water) in petri dishes, and placed in an incubator for 24 hr at 50 C. The seed coats were then easily removed and the embryos were planted into trays containing vermiculite using a template. Germination of F<sub>1</sub> embryos began 72 hr after planting, and within 144 hr, 94% of the seedlings had emerged. Two to 4% of the remaining seedlings emerged up to 1 week later. Trays were watered as needed with nutrient solution. This solution consisted of a Ca(NO<sub>3</sub>)<sub>2</sub> (3.0 mM), KNO<sub>3</sub> (1.0 mM), MgSO<sub>4</sub> (1.5 mM), K<sub>2</sub>SO<sub>4</sub> (1.25 mM), KH<sub>2</sub>PO<sub>4</sub> (2.5 mM), trace elements (1 ml stock solution/liter), and iron (1 ml stock solution/liter). The trace element stock solution contained H<sub>3</sub>BO<sub>4</sub> (2.86 g/liter), MnCl<sub>2</sub>·4H<sub>2</sub>O (1.81 g/liter), ZnSO<sub>4</sub>·7H<sub>2</sub>O (0.22 g/liter), CuSO<sub>4</sub>·5H<sub>2</sub>O (0.08 g/liter) and MoO<sub>3</sub> (0.07 g/liter). The iron stock solution contained 10 g sodium ferric diethylenetriamine pentaacetate (Geigy Sequestrene 330) per liter. Most seedlings were uniform at inoculation. The plants were incubated 3 weeks on a lighted bench (approximately 4,000 ft-c emitted by fluorescent lamps) in the laboratory, inoculated by a stem puncture method (2) at the three to four true-leaf stage, and placed in a growth chamber programmed to shift the temperature and light conditions according to the following schedule: (i) hold 20.5 C for 10 hr dark; (ii) gradually increasing from 20.5 to 24.5 C over a 5-hr period, with light intensities increasing to 4,000 ft-c in the first hr, beginning with 12, 100w incandescents followed sequentially in 30 min

TABLE 1. Numbers of  $F_1$  cotton plants from individual fruits of reciprocal A9519 (tolerant)  $\times$  A227 (susceptible) crosses which were tolerant or susceptible when inoculated with the nondefoliating SS-4 isolate of *Verticillium albo-atrum*<sup>a</sup>

Pedigree	Fruit No.	No. plants		Fruit No.	No. plants	
		Susceptible	Tolerant		Susceptible	Tolerant
A9519 <sup>b</sup>		2	30			
A227 <sup>b</sup>		26	0			
Apparently homozygous tolerant A9519 parental plants						
$F_1$ A227 $\times$ A9519	1	0	31	$F_1$ A9519 1 $\times$ A227		
$F_1$ A227 $\times$ A9519	3	1	20	$F_1$ A9519 3 $\times$ A227	0	16
$F_1$ A227 $\times$ A9519	5			$F_1$ A9519 5 $\times$ A227	0	10
$F_1$ A227 $\times$ A9519	6			$F_1$ A9519 6 $\times$ A227	0	14
$F_1$ A227 $\times$ A9519	7			$F_1$ A9519 7 $\times$ A227	0	14
$F_1$ A227 $\times$ A9519	10	0	11	$F_1$ A9519 10 $\times$ A227	0	17
$F_1$ A227 $\times$ A9519	11			$F_1$ A9519 11 $\times$ A227	0	21
$F_1$ A227 $\times$ A9519	12	0	14	$F_1$ A9519 12 $\times$ A227		
$F_1$ A227 $\times$ A9519	13	0	31	$F_1$ A9519 13 $\times$ A227	0	14
$F_1$ A227 $\times$ A9519	14			$F_1$ A9519 14 $\times$ A227	0	23
$F_1$ A227 $\times$ A9519	15	0	6	$F_1$ A9519 15 $\times$ A227	0	20
Apparently homozygous susceptible A9519 parent plant						
$F_1$ A227 $\times$ A9519	4	21	0	$F_1$ A9519 4 $\times$ A227	3	0
$F_1$ A227 $\times$ A9519	4	11	2			

<sup>a</sup> Plants were inoculated with  $10^6$  conidia/ml by a stem puncture method (3), and were incubated for 3 weeks in growth chambers programmed for 14-hr light (24.5 C) and 10-hr dark (20.5 C).

<sup>b</sup> From bulked selfed seed.

with 12 F96T12/VHO/CW lamps, to full intensity with the remaining 12 fluorescent lamps; (iii) hold 24.5 C for 7 hr light; (iv) gradually decreasing from 24.5 to 20.5 C over a 2-hr period, with lights shutting off in the reverse order.

In experiments 1 and 3, plants were inoculated with  $10^6$  conidia/ml of the SS-4 isolate; in experiment 2,  $10^6$  conidia/ml of the T-1 isolate were used. Inoculum was prepared from 3-day-old Czapek-Dox shake cultures inoculated with 4-mm mycelial plugs of the specific fungal isolate. Experiment 1 consisted of A9519, A227, and their  $F_1$  hybrids.  $F_1$  seed came from 12 A9519 plants reciprocally crossed with A227 plants. Pedigreed records of the A9519 plants were kept, but not those of A227, which my previous unpublished results established were homozygous-susceptible. A second part of experiment 1 consisted of plants of A9519, A227, and  $F_1$  hybrids; the  $F_1$  seed came from nine bolls selected randomly from approximately 600 A9519 plants crossed with A227 plants. Individual A9519 parent plants were not recorded, and each boll represented a different A9519 plant.

Experiment 2 had A9519, A227, and  $F_1$  plants from 10 individual bolls selected randomly from the 600 A9519 plants as explained above.

Experiment 3 consisted of plants of A1479, A227, and  $F_1$  plants from 24 bolls selected randomly from approximately 600 A1479 plants crossed with A227 plants. Seeds used to produce parental plants and control plants in the growth chamber were obtained from bulking selfed seed of the respective cotton strain.

RESULTS.—*Experiment 1.*—The A9519 parental plants expressed the tolerant reaction, showing mild chlorosis of older leaves with all new leaves symptom-

less. A227 plants expressed severe dwarfing and chlorosis of all foliage.  $F_1$  plants were either as tolerant as A9519 or as susceptible as A227. In Table 1, the numbers of tolerant and susceptible  $F_1$  plants are listed by the individual fruits and the related tolerant parent. A227 plants, when crossed with 11 A9519 plants, produced all tolerant  $F_1$  plants except one. The A227  $\times$  A9519-4 cross produced mostly susceptible  $F_1$  plants.

Table 2 shows the numbers of tolerant and susceptible A227  $\times$  A9519  $F_1$  plants obtained from seed of randomly selected bolls. Seed from eight bolls produced virtually all tolerant plants, while seed from one boll produced tolerant and susceptible plants in approximately equal numbers.

*Experiment 2.*—Plants of A9519, A227, and their  $F_1$  cross, when inoculated with the T-1 isolate, were completely defoliated 2 weeks after inoculation. Three weeks after inoculation, A9519 plants had produced regrowth at all nodes, but more prevalent at the lower nodes. The terminal buds were alive in most plants that showed little regrowth. Regrowth in 38% of the A227 plants was confined to the first and second node. Some of these plants had necrotic terminal regions. The remaining plants were alive without regrowth. The degree of regrowth in  $F_1$  plants, although variable, was similar to A9519 plants.

*Experiment 3.*—Most A1479 control plants inoculated with the SS-4 isolate expressed a tolerant response similar to that of A9519 plants. Many A1479 plants, classified as tolerant because of symptomless regrowth, were dwarfed. About 22% of the A1479 parental plants expressed a susceptible reaction. Table 3 shows tolerant and susceptible plants from the 24 bolls.

DISCUSSION.—The use of controlled environment and

TABLE 2. Numbers of F<sub>1</sub> cotton plants (fruits randomly selected from approximately 600, tolerant A9519 plants crossed with susceptible A227 plants) which were tolerant or susceptible when inoculated with the nondefoliating SS-4 isolate *Verticillium albo-atrum*<sup>a</sup>

Pedigree	No. plants	
	Susceptible	Tolerant
A9519 <sup>b</sup>	1	31
A227	23	0
A227 × A9519		
6 bolls	0	173
1 boll	1	27
1 boll	1	28
1 boll	18	14

<sup>a</sup> Plants were inoculated with 10<sup>6</sup> conidia/ml by a stem-puncture method (3), and were incubated for 3 weeks in a growth chamber programmed for 14-hr light (24.5 C) and 10-hr dark (20.5 C).

<sup>b</sup> From bulked selfed seed.

uniform plants provided an acceptable method of distinguishing differences between the tolerant A9519 and susceptible A227 control plants.

A model, using a dominant factor(s) which determines tolerance to the SS-4 isolate of *V. albo-atrum* in the A9519 strain, could explain the F<sub>1</sub> data. Assuming the 5% susceptible A9519 parental plants to be homozygous-recessive, one would expect homozygous- and heterozygous-tolerant plants within the A9519 strain. The A227 strain was considered homozygous-recessive because all plants were susceptible to the SS-4 isolate. In crosses of A227 plants to random A9519 plants, three F<sub>1</sub> responses would be expected: (i) Homozygous-dominant A9519 plants would produce all tolerant F<sub>1</sub> plants; (ii) heterozygous plants would produce tolerant and susceptible F<sub>1</sub> plants, in approximately equal numbers, assuming one gene; and (iii) homozygous-recessive plants would produce only susceptible F<sub>1</sub> plants. If the above assumption is correct, the A9519-4 plant in Table 1 may have been homozygous-recessive be-

TABLE 3. Numbers of F<sub>1</sub> cotton plants (fruits randomly selected from approximately 600, tolerant A1479 plants crossed with susceptible A227 plants) which were tolerant or susceptible when inoculated with the nondefoliating SS-4 isolate of *Verticillium albo-atrum*<sup>a</sup>

Pedigree	No. plants	
	Susceptible	Tolerant
A1479 <sup>b</sup>	10	45
A227 <sup>b</sup>	36	0
F <sub>1</sub> A227 × A1479		
20 bolls	205	390
3 bolls	0	93
1 boll	5	0

<sup>a</sup> Plants were inoculated with 10<sup>6</sup> conidia/ml by a stem-puncture method (3), and were incubated for 3 weeks in a growth chamber programmed for 14-hr light (24.5 C) and 10-hr dark (20.5 C).

<sup>b</sup> From bulked selfed seed.

cause of the susceptible F<sub>1</sub> progeny. Because most A9519 plants produced virtually all-tolerant F<sub>1</sub> plants, they were thought to be homozygous-dominant. The boll with 18 susceptible and 14 tolerant plants may have come from a heterozygous A9519 plant (Table 2). Data of F<sub>2</sub> progenies and progenies of F<sub>1</sub> plants backcrossed to the homozygous-susceptible A227 plants would provide more conclusive evidence of a single dominant gene determining tolerance to the SS-4 isolate in the A9519 strain. Occasional exceptions within fruits may be explained by inoculation difficulties or seed or pollen contamination. Only the three classes explained above occurred in the A227 × A9519 crosses.

The A227 × A9519 F<sub>1</sub> plants inoculated with the T-1 isolate expressed severe symptoms similar to the A9519 parent. Therefore, F<sub>1</sub> and parental responses of A227 and A9519 plants inoculated with SS-4 correlated to the severe isolate and to field responses.

Tolerance to the SS-4 isolate in the A1479 plants also appears to be dominant, but the F<sub>1</sub> differed from the A9519 F<sub>1</sub> response. The F<sub>1</sub> data (Table 3) indicate that a few A1479 plants may be either homozygous tolerant (3 bolls) or susceptible (1 boll), but most A1479 plants appeared to be heterozygous (20 bolls).

Another possibility is that a different temperature regime may be necessary for optimum phenotypic expression of the A1479 strain. Bell & Presley (1) showed that known levels of resistance in *Gossypium barbadense* were expressed at 25 C, but a tolerant *G. hirsutum* variety expressed a susceptible reaction.

These data clearly demonstrate the need of careful selection of homozygous parent plants before a genetic analysis is started. The bulking of F<sub>1</sub>, F<sub>2</sub>, or backcross seed obtained from heterozygous parental material would show a complex inheritance pattern, even if a single gene were segregating. The A9519 cotton strain appears to be good material for selecting homozygous parent plants. Other important factors to consider are the employment of careful crossing, selfing, and seed handling techniques. The progenies should then be analyzed under environmental conditions controlled to give maximum genotypic expression.

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