

### Critical Requirements for Genetic Expression of Verticillium Wilt Tolerance in Acala Cotton

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Halisky et al. (5) reported that the optimum temperature for *Verticillium albo-atrum* Reinke & Berth. in culture was 24 C, and severe *Verticillium* wilt symptoms were expressed in susceptible cotton when grown in infested soil maintained at 20 and 25 C. No symptoms developed when the soil temperature was 32 C. Berry & Thomas (3) found that the growth of *V. albo-atrum* was equally retarded at 10 C and 30 C in culture, but mint plants recovered with time at 30 C, while at 10 C the disease became increasingly severe. Bell & Presley (2) reported that a tolerant variety of cotton was classified as susceptible at mean greenhouse temperatures of 25 C, tolerant at 27 C, and resistant at 29 C, when it was inoculated with a defoliating isolate of *V. albo-atrum*.

My unpublished data show that symptoms were equally severe on Acala 9519, a tolerant cotton strain, and on Acala 227, a susceptible strain, when inoculated with a mild or severe isolate of *V. albo-atrum* in a greenhouse where temperatures averaged 18 C. Good differentiation between strains could be made when they were inoculated with the mild isolate at 25 C. Plants inoculated with the severe isolate at 25 C expressed symptoms too severe for good differentiation between strains. No symptoms developed in either strain, inoculated with either isolate at 35 C.

Susceptible A227 and tolerant A9519 cotton plants were grown, inoculated, and incubated in a growth chamber as previously described (1) to evaluate genetic differences between strains. Plants were stem-puncture inoculated (4) with three conidial concentrations of  $10^6$ ,  $10^4$ , and  $10^2$  conidia/ml of a severe

defoliating isolate, T-1, and a mild, nondefoliating isolate, SS-4, and placed in a growth chamber programmed for a 21.5 C night and a 27.0 C day temperature regime.

Table 1 shows the response of plants inoculated and grown under the above regime. As the concentration of inoculum increased, the percentage of plants expressing more severe symptoms increased. Symptom expression was too severe to distinguish accurately between A227 and A9519 plants inoculated with T-1 inoculum with  $10^6$  conidia/ml, although A9519 had slightly less symptom severity than A227. Symptom expression was slightly erratic in A9519 plants inoculated with T-1 at  $10^6$  conidia/ml, with 12% of the plants showing either mild or no symptoms. Symptom expression was too erratic in both cotton strains inoculated with SS-4 at  $10^6$  conidia/ml for accurate classification of all A227 and A9519 plants.

A second series of A227 and A9519 plants were grown and inoculated as described above with either SS-4 inoculum of  $10^6$  conidia/ml or T-1 inoculum of  $10^6$  conidia/ml, and grown in the growth chamber with a modified temperature regime of 20.5 C night and 24.5 C day. Table 2 shows more uniformity of symptom expression, except for plants showing no symptoms. These plants, being small at the time of inoculation, apparently escaped infection, possibly due to the restriction of conidial movement in the xylem vessels. Presley & Taylor (6) showed that end walls in the xylem vessels restricted conidial movement in very young plants, making them resistant to *Verticillium* infection. As these end walls dissolved, the plants became susceptible to infection. Plants showing no symptoms were reinoculated with the same isolate as the first inoculation at a more mature stage, and each plant developed symptoms characteristic of the preponderant number of plants in each treatment, indicating a primary "escape".

A227 and A9519 plants uniformly mature at the three-to-four true-leaf stage were inoculated, using only SS-4 inoculum at  $10^6$  conidia/ml and grown under the 20.5 C night and 24.5 C day regime in the growth chamber. Table 3 shows that A9519 plants expressed either a tolerant reaction similar to the field expression of A9519 (94%) or were as susceptible as A227 (6%).

TABLE 1. Percentages<sup>a</sup> of cotton plants (A9519, tolerant, and A227, susceptible) expressing various symptoms at 3 weeks after being inoculated with the defoliating T-1, or mild SS-4 isolate of *Verticillium albo-atrum* at 21.5 C (night) and 27 C (day)

Symptom expression	Conidia/ml/cotton strain					
	$10^6$		$10^4$		$10^2$	
	A9519	A227	A9519	A227	A9519	A227
	<i>Defoliating isolate (T-1)</i>					
No symptoms	4	0	14	0	38	26
Mild symptoms	8	0	7	5	0	13
Defoliated	88	100	79	95	62	61
	<i>Mild isolate (SS-4)</i>					
No symptoms	63	21	60	20	87	59
Mild symptoms	37	58	40	50	13	33
Severe chlorosis	0	21	0	30	0	8

<sup>a</sup> Based on approximately 25 plants/inoculation treatment.

TABLE 2. Percentages<sup>a</sup> of cotton plants (A9519, tolerant, and A227, susceptible) expressing various symptoms at 3 weeks after inoculation with 10<sup>6</sup> conidia/ml of the defoliating T-1 or mild SS-4 isolates of *Verticillium albo-atrum* at 20.5 (night) and 24.5 C (day)

Symptom expression	Fungal strain/cotton strain			
	T-1		SS-4	
	A9519	A227	A9519	A227
No symptoms	1 <sup>b</sup>	10 <sup>b</sup>	1 <sup>b</sup>	1 <sup>b</sup>
Mild symptoms	0	0	94	0
Severe chlorosis	0	0	5	99
Complete defoliation	99	90	0	0

<sup>a</sup> Based on 84 plants/inoculation treatment.

<sup>b</sup> Delayed germination resulted in small plants which apparently escaped infection. These plants expressed expected symptoms after a second inoculation.

Virtually all A227 plants (99%) expressed a uniform susceptible reaction. No escapes to infection were observed.

In a subsequent experiment, 154 A9519 and 128 uniformly mature A227 plants were inoculated at the three-to-four true-leaf stage with SS-4 inoculum of 10<sup>6</sup> conidia/ml and grown under a 20.5 C night and a 24.5 C day regime; 96.5% of A9519 plants expressed mild symptoms (tolerant), and 3.5% expressed severe symptoms similar to A227 plants. A227 plants were 99% susceptible and 1% tolerant.

In two other experiments with a 20.5 C night and 24.5 C day temperature regime, 32 A9519 and 32 A227

TABLE 3. Percentages<sup>a</sup> of cotton plants (A9519, tolerant, and A227, susceptible) expressing various symptoms at 3 weeks after inoculation with 10<sup>6</sup> conidia/ml of the SS-4 isolate of *Verticillium albo-atrum* at 20.5 C (night) and 24.5 C (day)

Symptom expression	Cotton strain	
	A9519	A227
No symptoms	0	0
Mild symptoms	94	1
Severe chlorosis	6	99

<sup>a</sup> Based on 190 plants/inoculated treatment.

plants were inoculated with SS-4 inoculum at 10<sup>6</sup> conidia/ml. Technical difficulties caused a rise in maximum day temperatures to 27 C for a 3-day period during the first week after inoculation. All A9519 plants in both experiments expressed a tolerant reaction (mild symptoms); 16 out of 32 susceptible A227 plants expressed a tolerant reaction in one experiment and 14 out of 32 A227 plants expressed a tolerant reaction in the second experiment.

Since all A9519 and A227 seed in each experiment came from the same bulked seed sources, these data indicate that maximum daily temperatures no greater than 24.5 C are necessary to separate genetic differences of the 2 strains when inoculated with the mild SS-4 isolate. Maximum day temperatures of 27 C allow genetically susceptible A227 plants to express an erroneous tolerant expression. These studies show that accurate classification of genetically tolerant and susceptible cotton plants was dependent upon carefully controlled temperature conditions, inoculum concentrations of 10<sup>6</sup> conidia/ml, and plants sufficiently mature at inoculation to allow free movement of conidia in the xylem vessels. Temperature requirements may change with different genetic materials to be evaluated.

#### LITERATURE CITED

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