Symptom Variability and Selection for Reduced Severity of Cotton Seedling Disease Caused by *Pythium ultimum*

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ABSTRACT

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Hypocotyls of seedlings of cotton cultivars Auburn M, Coker 310, Delcot 277J, and Dixie King 3 were inoculated with *Pythium ultimum*. Selected plants were grown to maturity and self-pollinated; progenies were evaluated for disease severity. Variation in hypocotyl symptom expression among seedlings within cultivars did not differ among the four cultivars. Variation in symptom expression among seedlings of Auburn M was not reduced significantly among progenies through three successive generations of selection and self-pollination. A small but significant difference in susceptibility to *P. ultimum* between Delcot 277J and Coker 310 was established. This difference was apparent in the parent seedlings and in two succeeding generations of selection and self-pollination of resistant plants. Reduced disease severity was demonstrated in progenies of Auburn M. Third-generation seedlings of Auburn M were significantly more resistant than the cultivar parent.

Major pathogens of cotton seedlings include *Rhizoctonia solani* Kühn, *Pythium* spp., *Thielaviopsis basicola* (Berk. & Br.) Ferr., and *Fusarium* spp. (2). These fungi cause a variety of symptoms including seed rot, preemergence and postemergence death of seedlings, root rot, and hypocotyl lesions (2,8). Present practices for control consist of in-furrow applications of fungicides and fungicide seed treatments to reduce seed rot. Because of the frequent occurrence of adverse post-planting weather conditions in Tennessee (3) and the diverse nature of the fungi that cause seedling diseases, fungicide applications often do not result in satisfactory control.

Resistance to the seedling disease complex is apparently not present in commercial cotton cultivars. Some of the multi-adversity-resistant lines selected at Texas A&M for cold tolerance have shown a measurable degree of resistance to *R. solani* (7). Johnson (1) demonstrated a small but significant difference in some commerical cultivars in disease susceptibility to *Pythium ultimum* Trow., one of the major pathogens in Tennessee. He noted much variation in symptom development among inoculated plants within cultivars.

The objectives of this study were to determine if variation in symptom expression among plants within a cultivar is genetic and if cultivar improvement in resistance to *P. ultimum* could be obtained with selection procedures.

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MATERIALS AND METHODS

Fungicide-treated seeds of cotton (*Gossypium hirsutum* L.) cultivars Coker 310, Dixie King 3, Delcot 277J, and Auburn M were obtained from P. E. Hoskinson, West Tennessee Experiment Station, Jackson. Seeds were planted in pots of soil in the greenhouse and grown to maturity. Plants were self-pollinated by stapling parchment corn shoot bags over squares (bracts surrounding unopened flower buds). Seeds were collected, bulked by cultivar, acid delinted, and used as "parent" seeds.

Parent seeds were planted in sterilized sand in plastic 10.2-cm pots (five seeds per pot) and incubated in continuous light in plant growth chambers at 28 C for 8 days. Pots were arranged randomly in groups of four, with each cultivar represented once per group. A total of 12 groups constituted an experiment. To reduce variability in seedling ages, which could affect susceptibility (1), only those that emerged 4–6 days after planting were used; seedlings that emerged 7 and 8 days after planting were removed and discarded. Usually, three seedlings per pot were retained for inoculation. On the eighth day after planting, hypocotyls of seedlings were inoculated (4) with a culture of *P. ultimum* designated B6-1 (ATCC 56081), originally isolated from a diseased seedling from a field in western Tennessee. A depression in the sand adjacent to each hypocotyl was made with a jet of water from a plastic wash bottle. A 5-mm-diameter agar disk cut with a cork borer from a 4- or 5-day-old culture of *P. ultimum* on potato-dextrose agar was placed with mycelium against the hypocotyl in the depression. Sand was pressed gently against the agar disk to hold it in place. Inoculated seedlings were removed after 7 days at 18 C and rated for disease severity on a scale of 0–5, where 0 = no visible symptoms; 1 = one to a few pinpoint dark spots or a faint diffused discolored area on the hypocotyl; 2 = a distinctly necrotic, usually sunken lesion less than 0.5 cm long; 3 = a lesion 0.5 cm or longer; 4 = plant wilted with cotyledons drooping; and 5 = dead plant. Tests of the parent seedlings were repeated five times. A total of 152 selected seedlings with ratings of 0, 1, 2, or 3 were transplanted individually into sterilized soil in pots, grown to maturity in the greenhouse, and self-pollinated for further study.

For determining disease severity, seeds from the self-pollinated plants were collected, acid delinted, and planted in pots of sterilized sand. Locations of pots and plants in pots were randomized within the growth chamber. One seedling from each self-pollinated parent was inoculated with *P. ultimum* and rated for disease severity in each growth chamber experiment. Thirty-eight parent plants of each of the four cultivars were represented in each experiment. Twelve such experiments were performed; thus a total of 12 progenies were evaluated from each self-pollinated parent along with 96 of each original cultivar parent.

Eight of the 152 self-pollinated plants whose progenies had the lowest disease index ratings were selected for further study. Auburn M, Delcot 277J, Coker 310, and Dixie King 3 were cultivar parents of one, one, three, and three of these selections, respectively. Progeny seedlings from these eight selections were inoculated; those with the lowest ratings were grown to maturity and self-pollinated. Eighteen seedlings from each of 26 self-pollinated plants were evaluated for disease severity in growth chamber experiments along with seedlings of the original cultivar parents. Four of the progenies with the lowest ratings were grown through another self-pollination generation. Progenies of this third generation were tested for disease severity along with original parent seedlings.

Analyses of variance of data were performed and treatment means separated with Duncan's new multiple range test. The response of the first generation was

calculated by the realized heritability method with data of progenies of parents whose disease index ratings were 1, using the formula $h^2 = R/i\sigma$, where R = the advance in one generation of selection, σ = the phenotypic standard deviation of the parental population, and i = selection intensity, a coefficient determined by the proportion of the population selected to be parents (5). With this method, the realized gain in disease resistance of first-generation progeny of each of the four cultivars was compared with that of the parent population of the same cultivar.

RESULTS

Disease severity ratings of parents and ratings of self-pollinated progenies and parent controls are listed in Table 1. In the initial tests of parents (parent generation), Delcot 277J was significantly more susceptible than the other three cultivars. In additional tests of parents used as controls for tests of progenies from self-pollinated parents, both Delcot 277J and Auburn M were more susceptible than Coker 310 but did not differ significantly from Dixie King 3. Likewise, the progenies of Delcot 277J and Auburn M were more susceptible than those of Coker 310.

The amount of variability in symptom expression among individual seedlings in a cultivar, as measured with standard deviation values, appeared to be transmitted through selfing to the progeny. Both parents and progeny of Coker 310 had the lowest standard deviation, whereas parents and progeny of Dixie King 3 had the highest.

As an average of all four cultivars, self-pollinated parents that differed in susceptibility yielded progenies that did not significantly differ in susceptibility (Table 2). Significant differences within some cultivars did occur. For example, progenies of Delcot 277J that had ratings of 0 were significantly less susceptible than progenies whose parents had ratings of 1, 2, or 3. Conversely, progenies of Auburn M parents with ratings of 0 were most susceptible. Auburn M progenies whose parents were rated 1 were the least susceptible. There was very little difference in progenies of Coker 310 or Dixie King 3 regardless of the parental rating. Standard deviations among progenies from parents of different ratings were similar.

An analysis of heritability was made with the realized heritability method (5) with data of progenies of parents that had disease index ratings of 1. Values obtained were Auburn M, 0.058; Coker 310, 0.012; Delcot 277J, 0.019; and Dixie King 3, −0.010. The realized gain in one generation was highest with progenies of Auburn M, but this gain was only 1.5%.

The results of a series of growth chamber tests of the second-generation progenies are given in Table 3. The only reduction in susceptibility was exhibited in progenies of plants whose original cultivar parent was Auburn M. This reduction, as an average of progenies of the seven selfed plants, did not represent statistical significance from parent susceptibility. Disease index means of progenies of the other cultivars were higher than those of their respective parents.

Third-generation progenies were the results of selections from four of the seven Auburn M second-generation plants. Seeds from plants within the four selection lines were bulked and seedlings tested for disease severity in four replicated tests along with seedlings of the original parent (Table 4). All lines were less susceptible than Auburn M at $P = 0.05$. The lines differed considerably in variability of symptom expression among individual plants. Mean standard deviation of all four lines was 1.10, a value comparable to that of the Auburn M parent.

DISCUSSION

When plants within a cotton cultivar are inoculated with *P. ultimum* by placing agar disks of mycelium against uninjured basal portions of the hypocotyls, symptoms can vary from none to death of the seedlings. Absence of symptoms or minor

Table 1. Susceptibility to *Pythium ultimum* of parent cotton cultivars and of progenies of self-pollinated selections[a]

Cultivar	Parent generation[b] (n = 152)	Progeny (n = 456)		Parent control (n = 96)	
		Mean[b]	SD[c]	Mean	SD
Auburn M	2.38 y	3.20 x	1.17	3.16 x	1.04
Coker 310	2.37 y	2.79 z	1.02	2.70 y	0.92
Delcot 277J	2.62 x	3.13 xy	1.11	3.14 x	1.10
Dixie King 3	2.26 y	3.00 y	1.29	2.94 xy	1.17

[a] Within each column, values followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.
[b] Based on a scale of 0–5, where 0 = no visible symptoms; 1 = one to a few pinpoint dark spots or a faint diffused discolored area on the hypocotyl; 2 = a distinctly necrotic, usually sunken lesion less than 0.5 cm long; 3 = a lesion 0.5 cm or longer; 4 = plant wilted with cotyledons drooping; and 5 = dead plant.
[c] Standard deviation based on individual plant ratings.

Table 2. Disease severity of progenies of four cultivars of self-pollinated cotton plants that differed in susceptibility to *Pythium ultimum*

Parent		Progeny		
Disease index[a]	No. of self-pollinated plants	No. of plants tested/parent	Mean disease index[a]	SD[b]
0	13	12	2.96	1.22
1	48	12	2.95	1.20
2	48	12	3.01	1.14
3	48	12	3.14	1.11

[a] Based on a scale of 0–5, where 0 = no visible symptoms; 1 = one to a few pinpoint dark spots or a faint diffused discolored area on the hypocotyl; 2 = a distinctly necrotic, usually sunken lesion less than 0.5 cm long; 3 = a lesion 0.5 cm or longer; 4 = plant wilted with cotyledons drooping; and 5 = dead plant.
[b] Standard deviation based on individual plant ratings.

Table 3. Susceptibility to *Pythium ultimum* of second self-pollinated generation cotton seedlings and of the original cultivar parents

Cultivar	No. selfed plants	Disease index[a]		
		Range	Mean	Mean difference[b]
Auburn M			3.15	
Progenies	7	2.23–3.62	2.81	0.34
Coker 310			2.92	
Progenies	6	3.30–4.10	3.67	−0.75
Delcot 277J			3.92	
Progenies	2	4.02–4.10	4.06	−0.14
Dixie King 3			2.92	
Progenies	6	3.30–4.10	3.67	−0.59

[a] Based on a scale of 0–5, where 0 = no visible symptoms; 1 = one to a few pinpoint dark spots or a faint diffused discolored area on the hypocotyl; 2 = a distinctly necrotic, usually sunken lesion less than 0.5 cm long; 3 = a lesion 0.5 cm or longer; 4 = plant wilted with cotyledons drooping; and 5 = dead plant.
[b] Difference between disease indices of the cultivar parent and progeny.

Table 4. Susceptibility to *Pythium ultimum* of self-pollinated third-generation cotton seedlings and the original parent, Auburn M[a]

Line or cultivar	Mean disease index[b,c]	SD[d]
1-8	2.50 x	1.17
1-10	2.63 xy	0.78
1-4	2.75 xy	1.45
1-12	3.05 y	0.98
Auburn M	3.60 z	1.20

[a] Results of four replicated tests made at different times.

[b] Based on a scale of 0–5, where 0 = no visible symptoms; 1 = one to a few pinpoint dark spots or a faint diffused discolored area on the hypocotyl; 2 = a distinctly necrotic, usually sunken lesion less than 0.5 cm long; 3 = a lesion 0.5 cm or longer; 4 = plant wilted with cotyledons drooping; and 5 = dead plant.

[c] Values followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's new multiple range test.

[d] Standard deviation based on individual plant ratings.

hypocotyl discoloration could occur if contact between the hypocotyl and mycelium is lost. However, we have often observed minor symptom development when intimate contact of inoculum and hypocotyl is maintained. An adjacent inoculated plant (in the same pot) that exhibits similar contact may be dead or have an extensive lesion.

Variation in epidermal cell wall thickness of the hypocotyls could be related, at least in part, to differences in susceptibility. In a previous study, Sutherland (6) did not find such a relationship. Epidermal cell wall thickness of 8-day-old hypocotyls ranged from 0.63 to 0.84 μm. Dixie King 3 cell walls averaged 0.76 μm and Delcot 277J averaged 0.72 μm, a difference that was not statistically significant; yet seedlings of Delcot 277J were more susceptible to *P. ultimum* than those of Dixie King 3. In our study, variation in symptoms among individual seedlings within a cultivar or line was measured by calculating standard deviations of disease index values. If variation in cell wall thickness is related to variation in symptom expression, then this characteristic was apparently not made more homogeneous by selecting and self-pollinating resistant plants through three generations.

In the third selfed generation, standard deviations differed among the four "resistant" lines, but the mean deviation of the four lines was similar to that of the cultivar parent. We believe that variation in symptom expression results from the inoculation procedure, which could produce slight injury to some of the hypocotyls, or slight differences in contact of hyphae with hypocotyls, or some factor or factors inherent in cotton seedlings not influenced by selection of plants resistant to *P. ultimum*.

This study confirms the existence of differences among cotton cultivars in susceptibility to *P. ultimum*. Previously, susceptibility of 27 cultivars was determined and compared with that of cultivar Stoneville 603 (1). Eight cultivars were more resistant and two were more susceptible than Stoneville 603. These differences were small but measurable. In our study, different sources of cultivar seed were used and similar significant differences were found among the cultivars, especially between Delcott 277J and Coker 310. The difference between the two cultivars was maintained through selection and self-pollination of resistant plants through two successive generations (Tables 1 and 3).

An indication that Auburn M might be the most likely cultivar to use for improvement in resistance was found through an analysis of progenies of cultivar parents that had disease index ratings of 1 with the realized heritability method. Although the realized heritability value for Auburn M represented only a very small gain in resistance, the value was higher than those of the other three cultivars. The analysis was verified by the results of two succeeding generations of selection and self-pollination. In the second generation, improvement in resistance was evident only in progenies of Auburn M. This improvement was confirmed and resistance was increased in the third generation through selection and self-pollination of the most resistant second-generation plants.

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