Identification of Resistance to *Thielaviopsis basicola* in Diploid Cotton

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ABSTRACT

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A screening program to find sources of resistance to *Thielaviopsis basicola* in cotton identified a diploid cotton (*Gossypium arboreum*, PI 1415) with apparent high resistance to this pathogen. Seedlings were obtained from self-pollinated seed taken from the resistant plant (PI 1415) and grown in growth chamber tests with the cultivars (tetraploid *G. hirsutum*) Paymaster HS-26 and Paymaster Tejas as controls. Seedlings were grown for 20 days in naturally infested field soil and evaluated for root growth and root necrosis. In all tests, PI 1415 had less ($P = 0.001$) root necrosis than the cultivars HS-26 and Tejas. A triadimenol seed treatment also resulted in less ($P = 0.001$) root necrosis for all three cotton genotypes, and the response was additive with level of disease resistance. Incorporation of the resistance factor from PI 1415 into tetraploid upland cotton may greatly reduce damage by black root rot, especially in combination with fungicide seed treatments.

Thielaviopsis basicola causes black root rot of cotton seedlings. Primary symptoms include necrosis of the tap and lateral roots that results in stunting and delay in crop maturity (4,7,13). Disease symptoms are most severe when growing conditions are not conducive for cotton growth. Temperature can greatly affect the severity of disease in cotton (4,18). Soil temperatures <26 to 28°C will increase the disease severity in cotton (4,18). Increasing soil moisture potential from -30 to -10 J/kg and decreasing soil temperature from 28 to 20°C resulted in an increase in the number of infections by *T. basicola* (18).

Resistance to black root rot has not been identified in upland cotton (23,24), and management of this disease has been accomplished by the use of crop rotation (primarily with grass species), flooding, and fungicide seed treatments (7). Crop rotation has not been used successfully in many locations, possibly due to the wide host range of this fungus (12) or extremely high fungal population densities. Fungicide seed treatments labeled for control of this disease include triadimenol and myclobutanil (1,5). Root necrosis on plants obtained from seed treated with these fungicides can be significantly reduced (2,8); however, with high levels of disease, these materials can be ineffective (15).

On the High Plains of Texas, over 1.2 million ha of cotton are planted each year

(21). Much of this region is at an altitude over 1,000 m, and only short-season cotton genotypes can be grown. Thus, cotton is often planted into cool soils, which is favorable for development of black root rot (4,8,13). Recommended control measures for black root rot do not provide sufficient disease control under these conditions. Therefore, a program was initiated in 1996 to screen cotton germ plasm for resistance to *T. basicola*. One diploid cotton line (*Gossypium arboreum*) with an apparent high level of resistance to black root rot was identified. The objective of this study was to determine the level of resistance present in PI 1415 to black root rot of cotton and whether the resistance was additive with fungicide seed treatments.

MATERIALS AND METHODS

Resistance screening. A program was initiated in 1996 to screen cotton germ plasm for resistance to black root rot. The screening procedure consisted of mixing 22,000 to 33,000 chlamydospores of *T. basicola* into 60 cm³ of sterilized soil (77% sand, 9% silt, and 14% clay), adding the infested soil to a plastic tube (2 cm diameter, 15 cm long), and planting a single seed that had been surface-sterilized with 30% H₂O₂ for 10 min and pregerminated in water agar. The cultivar (*Gossypium hirsutum*) Paymaster HS-26 was included in all tests as a susceptible standard. Cotton (5 seeds per genotype) was grown for 18 days at temperatures ranging from 16 to 22°C. The plants were then carefully removed from the tube, and roots were washed and visually rated for percent root necrosis.

Five tetraploid cotton lines and one diploid cotton line were identified as resistant in the primary screening (out of

approximately 1,000 cotton lines). Selections in the primary screening were required to have a 50% reduction in root necrosis in comparison to HS-26. These cotton seedlings were transplanted into 40-liter pots and grown to maturity. Seeds were collected from apparent resistant plants and retested using naturally infested field soil. The field soil, which was a sandy clay loam soil (60% sand, 17% silt, 22% clay, and 0.8% OM, pH 8.4), was initially tested for *T. basicola* density with dilution plating on TBCEN (20) and yielded *T. basicola* densities at 110 CFU/cm³ of soil. Twenty to 25 seeds of each genotype were planted in the field soil and grown for 20 days at 18°C. All emerging seed from the diploid cotton line had greater than 50% reduction in root necrosis compared with HS-26. The level of resistance in all the tetraploid cotton lines was much weaker in comparison. The diploid line was used in further experiments.

The field soil used in the previous section was further assayed for *Rhizoctonia solani* (16) and *Pythium* (14). Only low levels of *R. solani* were present in the soil. The most resistant line to *T. basicola* based on preliminary screening, PI 1415, was compared against HS-26, where the susceptible variety either was planted as fungicide free seed or with seed treated with a common fungicide combination (thiram + metalaxyl + triadimenol [0.82 + 0.14 + 0.10 ml a.i./kg of seed]) with activity against *Pythium*, *R. solani*, and *T. basicola*. Soil was collected from the field less than 1 week prior to testing, mixed, and then placed into Cone-Tainers (diameter 3.8 cm, length 14 cm; Stewe & Sons, Inc., Corvallis, OR) holding 110 cm³ of soil. A single seed was planted at a depth of 2.5 cm. Cone-Tainers were well watered and kept in a growth chamber at 17 to 18°C for 20 days. Roots were washed over a 0.86-mm pore sized sieve to catch roots. Total length of roots and the length of necrotic root tissue was measured on an image analysis system (DECAGON Devices, Inc., Pullman, WA). There were 25 replications of each treatment, and the test was repeated once. Both tests had a factorial arrangement of treatments in a randomized complete block design.

Integrated management tactics. A second field soil was identified with high levels of *T. basicola* (based on dilution plating on TBCEN) and low levels of other pathogens (primarily *R. solani*), based on soil assays (14,16) and cotton bioassays (9). Microscope examination of seedlings

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from the bioassays confirmed the presence of chlamydospores of *T. basicola* on necrotic root tissue. The soil had a clay texture (25% sand, 34% silt, 42% clay, and 2.1% OM, pH 8.2). Cotton genotypes tested were HS-26, Paymaster Tejas (*G. hirsutum*), and PI 1415. All three genotypes were tested with and without fungicide (thiram + metalaxyl + triadimenol [0.82 + 0.14 + 0.10 ml a.i./kg of seed]) seed treatments to determine the effectiveness of plant resistance with other types of disease protection. The experimental design was the same as in the previous section, except there were only eight replicates per treatment.

Data analysis. Analysis of variance was performed with PROC GLM (SAS Institute, Cary, NC) for total root length and percent necrotic roots. The percent necrotic roots was calculated by dividing the necrotic root length by total root length. There was an uneven number of replicates for root ratings, since only plants that emerged could be evaluated. Orthogonal contrasts were used to determine significant differences between susceptible and resistant varieties and between the cultivars.

RESULTS

Resistance screening. Results were similar for the two tests, so data are combined for presentation. Root necrosis for PI 1415 averaged 23% compared with 77% in HS-26 without fungicide seed treatment. Seed treatment reduced ($P = 0.001$) root necrosis on HS-26 from 77% to 47%

(Table 1). Root length was not affected by cotton genotype.

Integration of management tactics. Root necrosis was affected by seed treatment and cotton genotype. Roots of HS-26 and Tejas had more ($P = 0.001$) necrosis with seed treatment (63 and 65%, respectively) than roots of PI 1415 with seed treatment (17%) in test 1 (Table 2). Root necrosis of PI 1415 and Tejas with seed treatment was less ($P = 0.001$) (17 and 65%, respectively) than without seed treatment (47 and 81%, respectively) (Table 2). No plants emerged from the HS-26 seed without fungicide, so root length and root necrosis could not be measured. Root length was not affected by cotton genotype (with fungicides). When this experiment was repeated with another source of field soil, there were again no differences in root length between seed treatments or cotton genotypes. Root necrosis results were similar to the first test, with cotton genotype and seed treatment being significant with no interaction (Table 2). However, in the second test, seed of HS-26 without fungicide did emerge. Paymaster HS-26 and Tejas had significantly ($P = 0.001$) more root necrosis than PI 1415 (Table 2), and there was no difference between HS-26 and Tejas in terms of root necrosis (Table 2). There was more necrosis without seed treatment (62%) than with seed treatment (48%).

DISCUSSION

Management of black root rot in cotton is primarily by the use of seed treatments,

but current fungicides, although they have improved activity over earlier fungicides (7), are only partially effective (2,7,8,15). Black root rot in tobacco (*N. tabacum*) is controlled primarily through host resistance (19). High level of resistance in tobacco to *T. basicola* was initially discovered in *Nicotiana debneyi* (6), and low to moderate levels were identified in *N. tabacum* (6,25). Both sources of resistance have been used in tobacco production. Prior to the current study, there were no reports of resistance to black root rot in cotton, and extensive screening had demonstrated no differences in root symptoms among currently grown cultivars (23,24). The resistance identified in PI 1415 consistently resulted in less disease than found in HS-26 or Tejas in soils with high population densities of *T. basicola*. However, resistance is not immunity, and as conditions became more conducive to disease, root necrosis increased, even with PI 1415. Integrated control, which combined both plant resistance and fungicide seed treatment, resulted in superior disease control to either management tool used singly.

Resistance in tobacco has been shown to reduce lesion size, number, and secondary inoculum production of *T. basicola* (11). The mechanism of resistance in cotton has not yet been intensively studied, but it may yield similar information for lesion size and number. PI 1415 was crossed with susceptible *Gossypium herbaceum*, and the F_2 progeny were screened with soil naturally infested with *T. basicola*. The frequency distribution of root necrosis for the 200 F_2 progeny screened was similar to the resistant parent (T. A. Wheeler, unpublished). This indicates that resistance is dominant and probably involves one or a few genes.

Unfortunately, PI 1415, which was originally obtained from a tetraploid cotton collection, was identified as a diploid genotype of *G. arboreum* (David Stelly, personal communication). Cultivated upland cotton comes from the species *G. hirsutum* [$2n = 4x = 52$; genomic formula $2(AD)_1$]. *G. arboreum* has an A_2 type genome, which is often incompatible with *G. hirsutum* (3). Interspecific hybrids were obtained between *G. arboreum* and *G. hirsutum* (22), *G. anomalum* (10), and *G. stocksii* (10) through ovule embryo culture. The *G. arboreum* parent in one cross was described as resistant to root rot (22), although no proof was cited, nor was a disease organism listed. There are several routes that may be productive in moving the *T. basicola* resistance into upland cotton. One strategy would be to create interspecific hybrids with a D type genome such as *G. thurberi* ($2D_1$) and then double the chromosomes and cross the hybrid with *G. hirsutum*. Another strategy would be to have a detailed map of the location of the gene(s) (17) on *G. arboreum* so the piece of chromosome that carries the gene(s)

Table 1. Root necrosis on susceptible (Paymaster HS-26) and resistant (PI 1415) cotton when planted into a sandy clay loam soil naturally infested with *Thielaviopsis basicola*

Cotton type	ST ^w	% root necrosis ^x	n ^y	SD ^z
1415	—	23	31	18
HS-26	—	77	16	15
HS-26	+	47	32	27

^w Seed treatment with 0.85 ml of thiram + 0.14 ml of metalaxyl + 0.10 ml of triadimenol per kg of seed.

^x Represents the average from two tests.

^y Number of plants that emerged in the two tests.

^z Standard deviation.

Table 2. Mean and standard deviation (SD) of root necrosis on susceptible (Paymaster HS-26 and Tejas) and resistant (PI 1415) cotton with and without seed treatments when planted into a clay soil naturally infested with *Thielaviopsis basicola*

Cotton type	ST ^w	% root necrosis					
		Test 1			Test 2		
		Mean	SD	n ^x	Mean	SD	n
1415	—	47 b ^y	15	7	36 cd	21	8
1415	+	17 c	14	6	22 d	22	7
HS-26	—	— ^z	—	0	66 ab	18	7
HS-26	+	63 ab	23	5	59 bc	33	5
Tejas	—	81 a	14	6	86 a	15	8
Tejas	+	65 ab	26	7	64 ab	19	8

^w Seed treatment with 0.85 ml of thiram + 0.14 ml of metalaxyl + 0.10 ml of triadimenol per kg of seed.

^x Number of plants that emerged from the eight seeds planted.

^y Different letters indicate significant differences ($P = 0.05$) between treatments.

^z None of the seed emerged for this treatment combination.

could be moved into upland cotton. It will require substantial time and effort to transfer the resistance to tetraploid upland cotton. PI 1415 is the first known example of high black root rot resistance in cotton.

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LITERATURE CITED

1. Arthur, K. S. 1996. Baytan seed treatment fungicide: A review of field performance across the cotton growing regions. Page 269 in: Proc. Beltwide Cotton Conf. P. Dugger and D. A. Richter, eds. National Cotton Council, Memphis, TN.
2. Arthur, K. S., Hairston, W. G., and Graham, C. T. 1991. Utilizing triadimenol as a co-fungicide to enhance disease suppression of *Rhizoctonia* and *Thielaviopsis* in cotton stand establishment. Pages 169-172 in: Proc. Beltwide Cotton Conf. D. J. Herber, and D. A. Richter, eds. National Cotton Council, Memphis, TN.
3. Beasley, J. O. 1942. Meiotic chromosome behavior in species, species hybrids, haploids, and induced polyploids of *Gossypium*. Genetics 27:25-54.
4. Bland, L. J., Leyendecker, P. J., Jr., and Nakayama, R. M. 1953. Observations on black root rot symptoms on cotton seedlings at different soil temperatures. Plant Dis. Rep. 37:473-476.
5. Butler, L., Lawrence, D., and Becton, M. 1996. Nufrow M: A safe new seed treatment fungicide for the control of cotton seedling disease caused by *Thielaviopsis basicola* and *Rhizoctonia solani*. Pages 268-269 in: Proc. Beltwide Cotton Conf. P. Dugger and D. A. Richter, eds. National Cotton Council, Memphis, TN.
6. Clayton, E. E. 1969. The study of resistance to the black root rot disease of tobacco. Tob. Sci. 13:30-37.
7. DeVay, J. E., and Garber, R. H. 1997. Control approaches (cultural and chemical) for black root rot of cotton. Pages 79-83 in: Proc. Beltwide Cotton Conf. P. Dugger and D. A. Richter, eds. National Cotton Council, Memphis, TN.
8. Gannaway, J. R., Wheeler, T. A., Moore, J., Hake, K., Murphy, M., Schoenhals, L., and Coss, J. L. 1996. Cotton performance tests in the Texas High Plains and Trans-Pecos areas of Texas - 1995. Pages 179-188 in: Technical report 96-1. Texas Agricultural Experiment Station, College Station, TX.
9. Garber, R. H., DeVay, J. E., and Weinhold, A. R. 1982. Use of specific fungicides as tools to determine pathogens present in field soils. Pages 38-39 in: Proc. Beltwide Cotton Conf. J. M. Brown, ed. National Cotton Council, Memphis, TN.
10. Gill, M. S., and Bajaj, Y. P. S. 1986. In vitro production of interspecific hybrids in *Gossypium* and their utilization in backcrossing. Z. Pflanzenzüchtg. 96:174-176.
11. Hood, M. E., and Shew, H. D. 1996. Pathogenesis of *Thielaviopsis basicola* on a susceptible and a resistant cultivar of burley tobacco. Phytopathology 86:38-44.
12. Johnson, J. 1916. Host plants of *Thielavia basicola*. J. Agric. Res. 7:289-300.
13. Minton, E. B., and Garber, R. H. 1983. Controlling the seedling disease complex of cotton. Plant Dis. 67:115-118.
14. Mircetich, S. M. 1971. The role of *Pythium* in feeder roots of diseased and symptomless peach trees and in orchard soils in peach tree decline. Phytopathology 61:357-360.
15. O'Neill, G. 1997. Thielaviopsis - A grower's perspective. Page 75 in: Proc. Beltwide Cotton Conf. P. Dugger and D. A. Richter, eds. National Cotton Council, Memphis, TN.
16. Papavizas, G. C., Adams, P. B., Lumsden, R. D., Lewis, J. A., Dow, R. L., Ayers, W. A., and Kantzes, J. G. 1975. Ecology and epidemiology of *Rhizoctonia solani* in field soil. Phytopathology 65:871-877.
17. Reinisch, A. J., Dong, J., Brubaker, C. L., Stelly, D. M., Wendel, J. F., and Paterson, A. H. 1994. A detailed RFLP map of cotton, *Gossypium hirsutum* × *Gossypium barbadense*: Chromosome organization and evolution in a disomic polyploid genome. Genetics 138:829-847.
18. Rothrock, C. S. 1992. Influence of soil temperature, water, and texture on *Thielaviopsis basicola* and black root rot on cotton. Phytopathology 82:1202-1206.
19. Shew, H. D., and Shoemaker, P. B. 1993. Effects of host resistance and soil fumigation on *Thielaviopsis basicola* and development of black root rot on burley tobacco. Plant Dis. 77:1035-1039.
20. Specht, L. P., and Griffin, G. J. 1985. A selective medium for enumerating low populations of *Thielaviopsis basicola* in tobacco fields. Can. J. Plant Pathol. 7:438-441.
21. Texas Agricultural Statistics. 1994. U.S. Dep. Agric., Texas Dep. Agric., Austin, TX.
22. Thengane, S., Paranjpe, S. V., Khuspe, S. S., and Mascarenhas, A. F. 1986. Hybridization of *Gossypium* species through in ovulo embryo culture. Plant Cell, Tissue Organ Cult. 6:209-219.
23. Wang, H., and Davis, R. M. 1997. Susceptibility of selected cotton cultivars to seedling disease pathogens and benefits of chemical seed treatments. Plant Dis. 81:1085-1088.
24. Wheeler, T. A., and Gannaway, J. R. 1998. Effect of cotton pathogens on disease symptoms and yield of cotton varieties in large plot field trials. Pages 165-168 in: Proc. Beltwide Cotton Conf. P. Dugger and D. A. Richter, eds. National Cotton Council, Memphis, TN.
25. Wilkinson, C. A., Rufty, R. C., and Shew, H. D. 1991. Inheritance of partial resistance to black root rot in burley tobacco. Plant Dis. 75:889-892.