Research Project

Final Progress Report

Improving West Texas Cotton

Project Numbers: 04-518TX, 04-519TX, 04-520TX, 04-521TX, 04-522TX, and 04-531

Submitted

by the

West Texas Cotton Group

NON-TECHNICAL SUMMARY

Project Title: Improving West Texas Cotton

Principal Investigators: R.J. Wright[1,2], R. Allen[2], D. Auld[2], B. Efrem[2], J. Gannaway[1], and T. Wheeler[1]; [1]Texas Agricultural Experiment Station and [2]Texas Tech University

The emergence of new biotechnologies (commercial and public) and realization that future varieties will contain genes (transgenes) which enhanced drought tolerance has created a need to develop and learn the necessary know-how to accurately and confidently evaluate these technologies prior to commercialization. Texas cotton growers will need many questions answered regarding the economic, agronomic, and performance qualities of these technologies. This assessment will require a multi-disciplinary approach including plant breeders, an agronomist, pathologist, molecular geneticist, and molecular biologist.

The West Texas Cotton Group (WTCG) believes it is important to develop the know-how to accurately and confidently evaluate new biotechnologies prior to commercialization. The beneficial attributes and management of these new technologies is key information needed by Texas cotton growers. To assess our abilities to effectively evaluate these future technologies, we are testing available in-house drought tolerance technologies developed by the WTCG. Transgenic cotton plants that express catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) were developed by our group. These genes have been implicated in plant defense responses to stress. The efficacy of these technologies is being thoroughly evaluated in field, greenhouse, and laboratory experiments. To date, we have generated one year of replicated field testing of yield and fiber quality while a second year of testing is ongoing. The results from this study will be presented during the December meeting. Genetic tools and resources are also being developed to evaluate gene expression and support in the stacking of gene combinations.

The WTCG recently began (prior to the 2006 TSS project) to develop and evaluate new screening methods to assess a variety's resistance to important diseases. The 2006 project will concentrate on screening commercial varieties to the Verticillium wilt fungal pathogen. Varieties that are recommended based on low wilt ratings and high yields at a minimum of two sites include: Paymaster 2326RR, FiberMax (FM) 960BR, FM 989BR, FM 989B2R, and Deltapine 455BR. Two Roundup Ready Flex varieties that performed well at one site each are AFD 5064RF and AFD 5065B2F. These will be tested at more sites in 2007. Work is still underway on developing a screening technique in the greenhouse that will select between varieties with different levels of resistance to *V. dahliae*.The WTCG has led the way in using a team-based approach to successfully overcome several problems associated with abiotic stress and fiber quality. Initiated by the Texas State Support Committee in 2003, the WTCG has since successfully identified molecular markers diagnostic to Black Root Rot resistance, created methods to evaluate the performance of cotton lines grown under suboptimal (stress) conditions, and developed germplasm lines with superior fiber quality. An update of this progress and the teams plans to utilize our findings to benefit Texas cotton growers will be presented during the December meeting. The progress during the pass years has made possible the new phase of project goals present in this report.

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Principal Investigator:
Robert J. Wright[1, 2]
email: robert.wright@ttu.edu

Co-Principal Investigators:
Randy Allen[2]
E-mail: randy.allen@ttu.edu

Dick Auld[2]
E-mail: dick.auld@ttu.edu

Efrem Bechere[2]
E-mail: efrem.bechere@ttu.edu

John R. Gannaway[1]
E-mail: j-gannaway1@tamu.edu

Terry A. Wheeler[1]
E-mail: ta-wheeler@tamu.edu

[1]Texas Agricultural Experiment Station
Rt 3, Box 219
Lubbock, Texas 79403
Phone: (806) 746-6101
Fax: (806) 746-6528

[2]Texas Tech University
Dept of Plant and Soil Science
Box 42122
Lubbock, Texas 79409
Fax: (806) 742-0775

Performing Institution:
Texas Agricultural Experiment Station
Rt 3, Box 219
Lubbock, TX 79403

Texas Tech University
Lubbock, TX 79409

Responsible Contracts Officer:
Diane Gilliland
Assistant Director for Research Administration
Texas Agricultural Experiment Station
Jack K. Williams Administration Bldg
College Station, TX 77843
Phone: (979) 845-4761

Kathleen Harris
Associate Vice President for Research
Office of Research Services
Texas Tech University
Lubbock, Texas 79409-1035
Phone: (806) 742-3884
Fax: (806) 742-3892

PROJECT SUMMARY

In 2003, the Texas State Support Committee (TSSC) recommended a new strategy to fund research focusing on the genetic enhancement of cotton. The West Texas Cotton Group (WTCG) was created to more efficiently focus on key problems affecting Texas cotton producers. As its long-term mission, the WTCG is concentrating on problems affecting the stability of fiber production and quality. This model of group-oriented research has been extended to all areas of cotton research supported by the TSSC. The members of the WTCG are excited about this new collaborative emphasis and have firsthand witness its benefit. The WTCG has successfully identified several sources of resistance to the Black Root Rot fungus, developed germplasm lines with superior fiber quality, and has worked toward developing methods to evaluate the performance of cotton lines when grown under suboptimal (stress) conditions. Our progress during the pass two years has made possible the new phase of project goals present hereafter.

OBJECTIVES:

The specific objectives for this funding period are listed below followed by a detailed plan of work. These objectives are:

1. Develop a screening assay(s) to accurately and precisely differentiate cotton plants based on resistance to Verticillium wilt,
2. Integrate Black Root Rot resistance into Upland cotton,
3. Evaluate germplasm lines containing the CAT, APX, and GR transgenes, and screen obsolete race stocks and other wild cotton accessions for natural traits conveying drought and salt tolerance.
4. Create cotton lines with enhanced fiber quality

RESULTS AND PROGESS:

Objective 1: ***Develop a screening assay(s) to accurately and precisely differentiate cotton plants based on resistance to Verticillium wilt –*** Verticillium wilt incited by the fungal pathogen *Verticillium dahliae* can cause significant yield losses and lost revenue to Texas cotton growers. *Verticillium dahliae* requires cool temperatures and wet conditions during late July and/or August to incite disease symptoms. The environmental conditions during the 2004 and 2005 were ideal for the pathogen, resulting in increased yield losses. These ideal environmental conditions for the pathogen have not been seen since 1996. Due to the infrequent epidemic nature of *V. dahliae* it is difficult to accurately assess a variety's inherent level of resistance. This ultimately complicates providing any recommendations to the grower regarding potentially useful resistant varieties. At this point, there is a great need to find alternative screening methods to determine the disease response of currently grown varieties. It is equally important to develop a reliable and effective greenhouse screening assay to determine a varieties response to the Verticillium wilt pathogen independent of field epidemics. Therefore we propose the following steps to combat this disease.

1. Field testing of commercial and public varieties, and
2. Develop a greenhouse screening assay.

<u>Progress of Field Testing</u>

<u>Materials and Methods</u>

Six locations (Brownfield, Earth, Garden City, Levelland, Petersburg, and Slaton) were selected based on producer's observations of wilt in previous years. At each site, 32 to 36 varieties were planted in a randomized complete block design with four replications. Seeding rate was 4 seed/ft. of row, and plots were 35.5 ft. long and 2-rows wide. During planting, soil was taken (20 cores per sample, two samples) at each site and assayed for *V. dahliae*. The sites (Brownfield, Earth, Garden City, Levelland, Petersburg, and Slaton) were planted on 11 May, 9 June, 18 May, 8 May, 24 May, and 16 May, respectively. During August, incidence of wilt in the entire plot was counted. Five of the six sites were harvested with a 2-row John Deere Cotton Stripper (484 or 7445) which was capable of measuring plot weight with load cells inside the stripper basket (in a small cage area). A subsample was taken of the harvest from all plots in two of the four replications. The subsample was ginned to measure percent turnout, and lint was sent to the Texas Tech International Textile Center for HVI testing. The five sites (Brownfield, Earth, Levelland, Petersburg, and Slaton) were harvested on: 13 November, 18 December, 27 October, 2 November, and 31 October, respectively. The sixth site (Garden City) was not harvested because deer had fed on the plots.

The percent wilt was calculated by counting the number of plants with wilt symptoms in a plot and dividing by the total number of plants in the plot. The relative wilt rating was calculated by dividing the percent of wilt, by the highest average percent of wilt for a variety at that site. Yield was calculated by multiplying the harvest weight for a plot times the percent turnout, and then dividing by the acres ((71 ft. long x row width (ft)))/43560 ft. sq.). Relative yield was the yield divided by the highest average yield for a variety at that site. A best variety statistic was created that combined: (1-relative wilt)+ relative yield). Correlation analysis was used to relate HVI values to % wilt. Only factors that were significant at P=0.05 were presented. Linear regression was used to determine if there was a significant ($P \leq 0.05$) relationship between wilt and yield. If there was not, then data was not presented.

Results

Four of the six sites (Garden City, Levelland, Petersburg, and Slaton) had sufficiently high levels of *V. dahliae* in the soil at planting to cause substantial disease (Table 1). The Brownfield site had much lower levels, though this field had substantial wilt in 2005 when planted with Stoneville 4554B2RF. The Earth site did not have measurable levels of *V. dahliae* in the soil, and almost no disease developed during the season. The temperature was less conducive for Verticillium wilt in 2006 than in the previous two years. This may help to account for the low levels of wilt found in Brownfield in 2006 compared with the previous year. The Brownfield site averaged 5.5 microsclerotia/cc soil. The weather may affect the incidence and severity of wilt, and one objective is to better understand the relationship between density of microsclerotia, wilt incidence, and yield loss in the High Plains of Texas.

Table 1. Relationship between density of *Verticillium dahliae*in the soil and incidence of wilt.

Site	Average Microsclerotia per cc soil	Average Incidence of Wilt	Date of Wilt Rating
Earth	0.0	0.0	Sept. 8
Brownfield	5.5	1.8	Aug. 28
Petersburg	30.75	19.6	Aug. 18
Slaton	31.5	23.6	Aug. 24
Levelland	80.0	16.1	Aug. 8

Petersburg

There was a significant relationship between wilt ratings and yield (Fig. 1) at this site. The lowest wilt ratings were found with Bayer AFD 5065B2RF, Paymaster (PM) 2326RR, and Beltwide Cotton Genetics (BCG) 520R (Table 2). The highest wilt ratings were found with Beltwide (BW) 2038B2RF and FiberMax (FM) 9068RF (Table 2). The highest yield occurred with PM 2326RR. Also yielding well at this site was FM 989RR, BCG 520R, AFD 5065B2RF, NexGen (NG) 2448R, and PM 2280BR (Table 2). Varieties that yielded poorly included: ST 4554B2RF, DP 110RF, All-Tex (AT) Atlas RR, ST 4700B2RF and ST 4664RF (Table 2). The combined statistic of relative wilt and relative yield resulted in four recommended varieties: PM 2326RR, AFD 5065B2RF, BCG 520R, and FM 989RR. Yield was multiplied by the loan value, and then the cost of the seed and technology fees (www.plainscotton.org/Seed/seedindex.html) were subtracted. One variety, PM 2326RR (Table 2,

$704.15/acre), was clearly the best variety that could have been planted at that site, among those tested. Loan values were highest for ST NG 2448R ($0.538/lb) and PM 2326RR ($0.522) (Table 2). Cotton from this field had very low values of micronaire overall (Table 3) and varieties with the highest micronaire also had the highest loan values. Wilt incidence was negatively correlated with micronaire (r = -0.45), uniformity (r = -0.54), and loan value (r = -0.49).

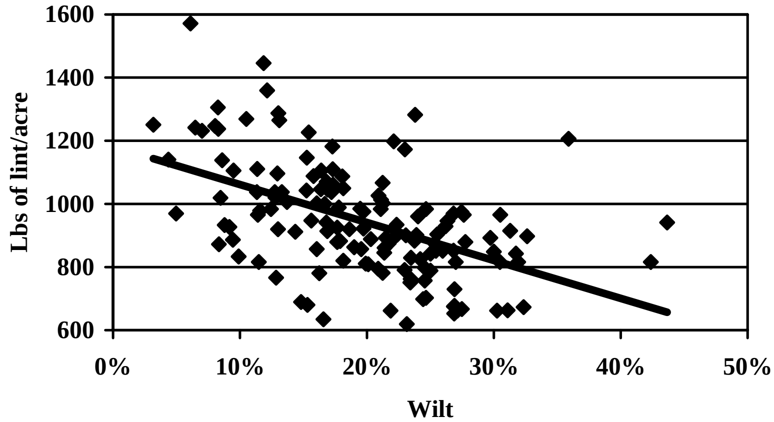


Figure 1. Relationship between wilt incidence on August 18 and yield in a field near Petersburg, TX in 2006. The regression equation fitted to the points is Lbs of lint/acre = 1,181 –12(% Wilt), R^2 = 0.26.

Table 2. Variety performance at a Verticillium wilt site near Petersburg, TX in 2006.

Variety	(1-relative wilt) + relative yield[a]	Lbs of lint per acre	% Wilt on 8/18/06	Loan Value ($)	$/acre[b]
Paymaster 2326RR	1.62 a[c]	1,400 a	11 fg	0.522	704.15 a
AFD 5065B2RF	1.46 ab	1,145 bcd	11 g	0.485	521.54 bcd
Beltwide Cotton Genetics 520R	1.38 abc	1,177 bc	14 efg	0.517	537.33 b
FiberMax 989RR	1.35 abc	1,215 b	16 c-g	0.488	547.63 b
Stoneville NexGen 2448R	1.24 bcd	1,102 b-d	16 b-g	0.538	542.11 b
Paymaster 2167R	1.22 b-e	1,046 def	16 c-g	0.510	452.48 cde
Stoneville 5242BR	1.21 b-f	1,030 d-g	16 c-g	0.478	432.82 efg
FiberMax 989B2R	1.21 b-f	1,026 d-h	16 c-g	0.474	438.17 def
Paymaster 2266RR	1.19 b-g	1,091 cde	17 b-g	0.516	530.49 bc
DynaGro 2100B2RF	1.19 b-g	946 f-j	14 d-g	0.451	370.90 e-k
Stoneville NexGen 1553R	1.10 c-h	1,020 e-h	19 b-g	0.486	401.80 e-i
Beltwide 3255B2RF	1.09 c-h	906 h-m	17 b-g	0.456	370.71 e-k
Paymaster 2280BR	1.09 c-h	1,118 b-e	21 a-e	0.478	428.71 efg
FiberMax 9063B2RF	1.09 c-h	913 g-l	17 b-g	0.463	362.06 f-k
Cropland Genetics 3020B2RF	1.08 c-h	896 i-n	17 b-g	0.460	352.61 g-l
FiberMax 9060RF	1.04 d-i	1,004 e-i	20 b-f	0.464	407.68 e-h
Stoneville 4664RF	1.03 d-j	791 mno	16 c-g	0.465	325.10 h-m
DeltaPine 424B2R	1.01 d-k	817 k-o	17 b-g	0.473	309.51 j-n
FiberMax 960RR	1.00 d-k	921 g-l	20 b-g	0.448	370.34 e-k
Beltwide 4021B2RF	0.97 d-k	894 i-n	20 b-f	0.432	308.55 j-n
FiberMax 960BR	0.93 e-k	932 f-k	22 a-e	0.505	419.61 efg
Stoneville 4700B2RF	0.92 e-k	785 no	19 b-g	0.433	277.90 lmn
FiberMax 960B2R	0.92 f-k	947 f-j	23 a-e	0.470	376.73 e-j
Beltwide Cotton Genetics 50R	0.91 g-k	937 f-j	23 a-e	0.456	364.84 f-k
Cropland Genetics 3520B2RF	0.88 h-l	848 j-o	22 a-e	0.447	324.97 h-m
FiberMax 9058RF	0.85 h-m	934 f-k	24 abc	0.452	356.25 f-l
All-Tex Atlas RR	0.84 h-m	783 no	21 a-e	0.442	297.08 j-n
Stoneville 4357B2RF	0.84 h-m	891 i-n	24 abc	0.448	322.71 i-m
All-Tex Summitt B2RF	0.81 h-m	817 k-o	23 a-d	0.459	320.66 i-m
DeltaPine 117B2RF	0.76 i-m	806 l-o	24 abc	0.393	234.24 n
Stoneville 4554B2RF	0.74 j-m	755 o	24 abc	0.446	327.07 h-m
DynaGro 2242B2RF	0.73 j-m	817 k-o	25 ab	0.419	265.55 mn
DeltaPine 110RF	0.72 klm	753 o	24 abc	0.428	287.63 k-n
FiberMax 9068RF	0.60 lm	834 j-o	30 a	0.455	310.69 j-n
Beltwide 2038B2RF	0.57 m	805 l-o	30 a	0.439	278.42 lmn

[a]This measure is an attempt to combine both the wilt ratings and yield to determine the best varieties to grow in Verticillium wilt fields. The higher the numbers are the better varieties for Verticillium wilt fields. Relative wilt is the percent wilt, divided by the highest average wilt found in any variety. Relative yield is the yield divided by the highest average yield for any variety.
[b]The $/acre was calculated by: (Yield (lbs/a) x loan value ($/lb)), - (seed and technology fees $/acre), obtained from the Plains Cotton Growers (www.plainscotton.org/Seed/seedindex.html).
[c]Letters that are different indicate that the mean values were significantly different at P=0.05.

Table 3. Fiber properties of varieties in a Verticillium wilt field near Petersburg, TX.

Variety	Mic[a]	Length	Unif.	Stren.	Elon.	Leaf	Rd	+b
AFD 5065B2RF	2.70	1.17	81.2	27.4	7.1	3.5	81.5	7.3
All-Tex Atlas RR	2.35	1.10	80.6	27.7	6.6	3.5	80.6	8.1
All-Tex Summitt B2RF	2.55	1.14	80.0	23.8	6.8	3.5	81.3	7.8
Beltwide 2038B2RF	2.45	1.16	79.8	24.5	7.0	4.0	80.0	7.8
Beltwide 3255B2RF	2.40	1.12	80.2	24.0	6.9	3.0	81.7	8.0
Beltwide 4021B2RF	2.15	1.14	79.1	22.3	6.9	3.5	81.6	7.4
Beltwide Cotton Genetics 50R	2.45	1.11	80.3	27.1	7.0	3.5	81.0	7.9
Beltwide Cotton Genetics 520R	3.10	1.14	82.5	27.8	6.6	3.5	80.7	7.8
Cropland Genetics 3020B2RF	2.35	1.13	80.1	24.2	6.8	3.0	82.0	8.1
Cropland Genetics 3520B2RF	2.65	1.15	79.5	23.9	7.3	4.0	80.4	7.7
DeltaPine 110RF	2.40	1.15	81.3	29.2	7.3	5.0	77.7	7.9
DeltaPine 117B2RF	2.25	1.16	79.3	26.5	6.0	5.5	77.5	7.6
DeltaPine 424B2R	2.55	1.13	81.1	25.0	7.1	3.5	81.6	8.0
DynaGro 2100B2RF	2.35	1.13	80.8	24.6	7.1	3.0	81.8	8.0
DynaGro 2242B2RF	2.35	1.14	78.6	23.0	7.5	4.0	79.9	7.8
FiberMax 9058RF	2.45	1.20	79.5	26.9	6.1	3.5	81.3	7.3
FiberMax 9060RF	2.40	1.20	79.6	26.9	6.0	3.0	82.5	7.5
FiberMax 9063B2RF	2.35	1.21	80.5	29.0	6.1	3.5	82.7	7.3
FiberMax 9068RF	2.40	1.19	80.3	29.4	6.5	4.0	81.7	7.4
FiberMax 960B2R	2.35	1.15	79.3	26.8	5.2	3.0	82.8	7.4
FiberMax 960BR	2.60	1.11	81.1	29.3	5.5	3.0	82.1	7.5
FiberMax 960RR	2.30	1.16	80.0	28.3	5.6	4.0	82.3	7.4
FiberMax 989B2R	2.35	1.18	81.6	28.7	5.8	3.0	82.5	7.7
FiberMax 989RR	2.55	1.15	81.0	29.1	5.7	3.0	83.0	7.8
Paymaster 2167R	3.25	1.06	82.3	27.0	7.7	3.5	80.6	8.1
Paymaster 2266RR	3.00	1.12	82.7	29.0	7.0	4.0	80.1	7.8
Paymaster 2280BR	2.60	1.13	81.3	28.1	6.4	3.5	80.0	8.0
Paymaster 2326RR	3.25	1.12	82.7	28.3	7.6	4.0	79.1	8.1
Stoneville 4357B2RF	2.35	1.14	78.7	23.2	7.0	3.0	81.0	8.1
Stoneville 4554B2RF	2.50	1.15	80.4	26.4	7.7	4.0	79.4	8.5
Stoneville 4664RF	2.60	1.14	81.1	26.1	7.7	4.0	79.5	8.3
Stoneville 4700B2RF	2.40	1.16	79.3	24.3	7.2	4.0	80.9	7.9
Stoneville 5242BR	2.55	1.10	80.5	24.9	6.8	2.5	82.0	8.7
Stoneville NexGen 1553R	2.70	1.18	81.2	28.4	7.0	3.5	81.0	7.6
Stoneville NexGen 2448R	3.15	1.13	82.5	27.8	6.9	3.0	80.6	7.9

[a]Micr=micronaire, length is in hundreths of an inch, stren=strength in grams/tex, unif.=uniformity in percentage, Elong.=elongation is the amount that a fiber will stretch prior to breakage, Leaf=leaf grade, Rd=degree of reflectance which is how light or dark the fiber is where grayer samples have lower values, +b=yellowness, where higher values indicate yellower samples.

Levelland

There was a significant relationship between wilt ratings taken on August 8 and yield (Fig. 2). PM 2326RR had much lower wilt ratings at this site than the other varieties (Table 4). The variety with the highest incidence of wilt at this site was DP 454BR (Table 4). Yield was highest for FM 960BR, FM 960B2R, and DP 455BR (Table 4). Yield was lowest for PhytoGen (PG) 370WR and AT Atlas RR (Table 4). The combined statistic of relative wilt and relative yield resulted in six recommended varieties: PM 2326RR, DP 455BR, FM 960BR, FM 960B2R, AT Patriot RR, and FM 989BR (Table 4). The top two valued varieties ((yield/acre X loan value) minus seed and technology costs)) were FM 960BR and PM 2326RR (Table 4). Micronaire was also low overall at this site (Table 5). Wilt incidence was negatively correlated to micronaire (r = -0.48), uniformity (r = -0.25), degree of reflectance (r = -0.32), and loan value (r = -0.50), and positively associated with leaf grade (r = 0.30).

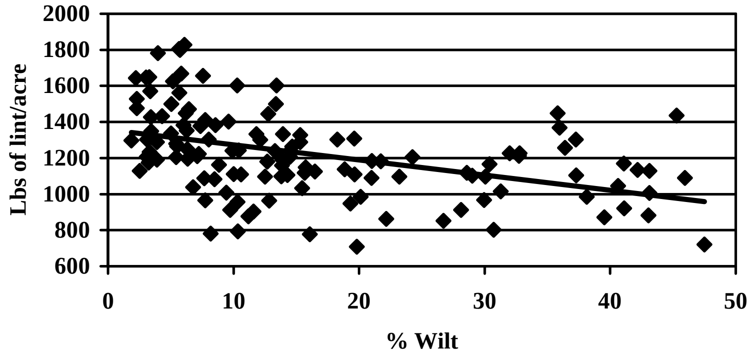


Figure 2. Relationship between wilt incidence on August 8 and yield at a field near Levelland, TX in 2006. The regression equation fitted to the points is Lbs of lint/acre = 1,358 –8(% Wilt), R^2 = 0.19.

Table 4. Variety performance at a Verticillium wilt site near Levelland, TX in 2006.

Variety	(1-relative wilt) + relative yield[a]	Lbs of lint per acre	% Wilt on 8/8/06	Loan values ($)	$/acre[b]
Paymaster 2326RR	1.59 a[c]	1,520 bc	7 c	0.585	860.91 ab
DeltaPine 455BR	1.43 ab	1,597 ab	12 bc	0.539	807.17 b
FiberMax 960BR	1.37 abc	1,668 a	15 abc	0.564	895.42 a
FiberMax 960B2R	1.34 a-d	1,599 ab	14 abc	0.544	824.32 b
All-Tex Patriot RR	1.34 a-d	1,364 de	14 bc	0.531	693.50 cd
FiberMax 989BR	1.32 a-e	1,462 cd	13 bc	0.528	725.51 c
DynaGro 2520B2F	1.22 b-f	1,378 de	14 bc	0.494	625.16 efg
NexGen 3273B2F	1.19 b-g	1,207 fgh	12 bc	0.513	565.02 ghi
All-Tex Apex B2F	1.18 b-g	1,376 de	15 abc	0.506	640.09 de
Stoneville 5007 B2F	1.14 b-g	1,124 hij	12 bc	0.514	521.47 ij
FiberMax 960RR	1.13 b-g	1,352 de	16 abc	0.518	662.45 cde
PhytoGen 125F	1.07 c-h	1,171 f-i	15 abc	0.524	567.16 f-i
Cropland Genetics 4020B2F	1.06 c-h	1,194 fgh	15 abc	0.514	556.51 hi
All-Tex 55066B2F	1.05 c-h	1,122 hij	14 abc	0.496	not sold in 2006
Beltwide 9775B2F	1.04 c-h	1,136 g-j	15 abc	0.512	526.98 ij
Americot 427R	1.03 c-g	1,174 f-i	16 abc	0.499	560.03 ghi
PhytoGen 480WR	1.03 d-h	1,377 de	18 ab	0.527	672.87 cde
All-Tex 45014F	0.98 e-h	981 k	14 bc	0.531	not sold in 2006
Beltwide 3552B2F	0.97 fgh	971 k	14 bc	0.444	376.25 mn
All-Tex 45039B2F	0.95 f-i	1,169 f-i	17 ab	0.527	not sold in 2006
Beltwide 4630B2F	0.94 f-i	1,219 fgh	18 ab	0.509	565.69 f-i
All-Tex Marathon B2F	0.94 f-i	1,256 efg	19 ab	0.500	570.86 f-i
PhytoGen 425F	0.90 f-j	1,273 ef	20 ab	0.534	632.31 def
All-Tex Titan B2F	0.88 g-j	1,119 hij	18 ab	0.475	475.06 jk
All-Tex Atlas RR	0.87 g-j	844 lm	15 abc	0.522	414.84 klm
Stoneville NexGen 3550F	0.87 g-j	1,173 f-i	19 ab	0.474	516.16 ij
Americot 1532B2F	0.87 g-j	1,204 fgh	20 ab	0.474	518.19 ij
PhytoGen 485WF	0.86 g-j	1,261 ef	21 ab	0.536	621.66 e-h
Beltwide Cotton Genetics 50R	0.77 hij	955 kl	19 ab	0.495	444.99 kl
Beltwide 3220B2F	0.77 hij	1,063 ijk	20 ab	0.495	470.99 jk
DeltaPine 454BR	0.61 ij	1,017 jk	23 a	0.447	403.03 lmn
PhytoGen 370WR	0.58 j	815 m	21 ab	0.488	344.91 n

[a]This measure is an attempt to combine both the wilt ratings and yield to determine the best varieties to grow in Verticillium wilt fields. The higher the numbers are the better varieties for Verticillium wilt fields. Relative wilt is the percent wilt, divided by the highest average wilt found in any variety. Relative yield is the yield divided by the highest average yield for any variety.
[b]The $/acre was calculated by: (Yield (lbs/a) x loan value ($/lb)), - (seed and technology fees $/acre), obtained from the Plains Cotton Growers (www.plainscotton.org/Seed/seedindex.html).
[c]Letters that are different indicate that the mean values were significantly different at *P*=0.05.

Table 5. Fiber properties of varieties at a Verticillium wilt field near Levelland, TX in 2006.

Variety	Mic[a]	Length	Unif	Stren	Elon	Leaf	Rd	+b
All-Tex 45014F	3.50	1.12	82.6	33.3	7.0	4.0	78.8	7.3
All-Tex 45039B2F	3.10	1.12	80.7	31.0	6.3	3.5	80.5	7.3
All-Tex 55066B2F	2.70	1.14	79.6	25.9	7.3	3.0	81.0	7.6
All-Tex Apex B2F	2.90	1.14	79.6	26.1	6.9	3.0	81.8	7.8
All-Tex Atlas RR	3.00	1.13	82.2	32.3	6.8	3.5	81.2	7.7
All-Tex Marathon B2F	2.90	1.13	78.8	24.7	7.1	3.0	82.1	7.7
All-Tex Patriot RR	3.05	1.15	80.3	27.5	6.9	2.5	83.0	7.8
All-Tex Titan B2F	2.85	1.17	79.4	28.0	6.8	4.0	80.1	7.3
Americot 1532B2F	2.80	1.12	78.8	25.5	6.6	3.5	81.4	7.7
Americot 427R	2.90	1.09	79.4	26.6	6.6	3.0	81.2	7.8
Beltwide 3220B2F	2.75	1.15	81.3	30.7	6.3	3.5	82.0	6.8
Beltwide 3552B2F	2.50	1.14	78.8	29.5	6.4	4.0	81.1	7.0
Beltwide 4630B2F	2.80	1.13	79.0	26.4	7.1	3.0	81.7	7.7
Beltwide 9775B2F	3.05	1.19	80.9	27.2	7.0	3.5	82.1	7.6
Beltwide Cotton Genetics 50R	2.75	1.11	80.2	28.5	6.9	3.0	82.5	7.8
Cropland Genetics 4020B2F	2.95	1.14	80.5	25.8	6.7	3.0	81.4	7.9
DeltaPine 454BR	2.50	1.09	79.5	27.2	6.2	4.0	81.1	7.1
DeltaPine 455BR	3.20	1.14	80.0	30.5	5.9	3.0	81.0	8.5
DynaGro 2520B2F	2.95	1.16	79.7	25.7	6.8	3.5	81.5	7.7
FiberMax 960B2R	3.25	1.14	79.6	29.3	5.6	2.5	82.7	7.7
FiberMax 960BR	3.30	1.13	80.9	33.0	5.8	3.0	83.1	7.5
FiberMax 960RR	2.85	1.15	80.2	30.3	5.6	3.0	82.5	7.3
FiberMax 989BR	3.15	1.14	80.8	29.5	5.9	3.0	83.2	7.5
Paymaster 2326RR	4.05	1.11	83.5	29.9	7.0	3.0	80.6	8.3
PhytoGen 125F	3.35	1.11	83.2	32.4	6.7	4.0	80.1	7.4
PhytoGen 370WR	2.75	1.09	80.2	26.8	6.8	3.5	81.3	8.0
PhytoGen 425F	3.35	1.15	81.7	29.0	7.4	4.0	79.3	8.1
PhytoGen 480WR	3.10	1.15	82.0	28.2	7.8	3.5	80.2	7.9
PhytoGen 485WF	3.45	1.14	82.0	27.7	7.7	4.0	79.3	8.2
Stoneville 5007 B2F	2.80	1.20	80.8	27.9	6.8	3.0	81.9	7.4
Stoneville NexGen 3273B2F	2.90	1.12	80.1	24.8	7.0	3.0	82.9	7.5
Stoneville NexGen 3550F	3.00	1.14	80.0	29.4	7.2	4.0	79.8	7.2

[a]Micr=micronaire, length is in hundreths of an inch, stren=strength in grams/tex, unif.=uniformity in percentage, Elong.=elongation is the amount that a fiber will stretch prior to breakage, Leaf=leaf grade, Rd=degree of reflectance which is how light or dark the fiber is where grayer samples have lower values, +b=yellowness, where higher values indicate yellower samples.

Slaton

There was a significant relationship between Verticillium wilt incidence and yield at this site (Fig. 3). Incidence of wilt at this site was lowest for FM 832LL, AFD 5064RF, PG 440W, and AFD Raider 271 (Table 6). Wilt incidence was highest for Cropland Genetics (CG) 4020B2RF, Americot (AM) 8120, BCG 295, and FM 958 (Table 6). Yield was highest for FM 960BR, FM 988LLB2, PG 440W, AFD 5064RF, FM 958LL, FM 989BR, and AFD Raider 271 (Table 6). Yields were lowest for FM 832, DP 393, BCG 295, BCG 50R, and CG 4020B2RF (Table 6). Plant stands were low at this site for most varieties, and were significantly correlated with incidence of wilt (R^2=0.24) and yield (R^2=0.18). Varieties with the highest combined yield and wilt ratings included: AFD 5064RF, FM 832LL, PG 440W, AFD Raider 271, and FM 988LLB2 (Table 6). The HVI ratings were not available for this site at the time this report was submitted.

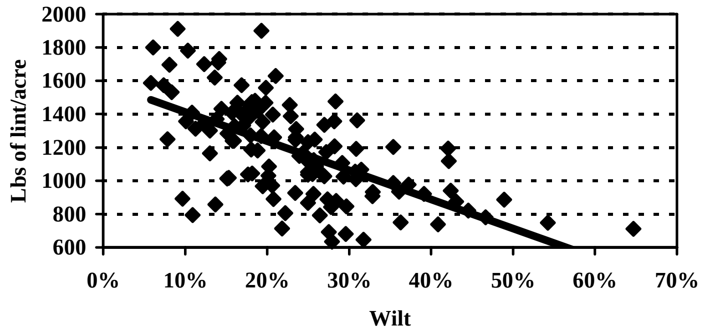


Figure 3. Relationship between wilt incidence on August 24 and yield at a field near Slaton, TX in 2006. The regression equation fitted to the points is Lbs of lint/acre = 1,587 –17.5(% Wilt), R^2 = 0.38.

Table 6. Variety performance at a Verticillium wilt site in Slaton, TX in 2006.

Variety	(1-relative wilt) + relative yield[a]	Lbs of lint per acre	% Wilt on 8/24/06	Plants/ ft. row
AFD 5064RF	1.66 a[b]	1,510 ab	10.8 hi	1.8 a-g
FiberMax 832LL	1.60 ab	1,369 a-d	9.7 i	2.3 ab
PhytoGen 440W	1.58 abc	1,514 ab	13.8 ghi	2.4 a
AFD Raider 271	1.50 a-d	1,461 ab	15.3 f-i	2.1 abc
FiberMax 988LLB2	1.50 a-d	1,518 ab	16.8 e-i	1.9 a-f
FiberMax 958LL	1.46 a-e	1,478 ab	17.4 e-i	2.0 a-d
NexGen 2448R	1.45 a-f	1,442 abc	16.9 e-i	1.9 a-f
FiberMax 960BR	1.45 a-f	1,587 a	20.4 d-i	1.7 c-i
FiberMax 989BR	1.45 a-f	1,473 ab	17.7 e-i	2.1 abc
FiberMax 966LL	1.27 a-g	1,340 b-e	21.3 c-i	1.7 c-i
DynaGro 2242B2RF	1.24 a-h	1,154 def	17.9 e-i	1.6 c-i
PhytoGen 125F	1.24 a-i	1,156 def	18.2 e-i	1.6 c-i
FiberMax 981LL	1.16 b-j	1,215 c-f	22.5 b-i	1.8 a-f
FiberMax 5035LL	1.15 b-j	1,215 c-f	22.5 b-i	1.8 b-h
FiberMax 965LLB2	1.14 c-j	1,195 def	22.7 b-i	1.3 ghi
Deltapine 117B2RF	1.08 d-j	1,090 fgh	22.5 b-i	1.9 a-f
AFD 5062LL	1.02 e-j	1,159 def	26.2 a-g	1.8 a-g
Beltwide Cotton Genetics 245	1.00 f-l	1,019 f-i	23.8 a-h	1.4 d-i
Beltwide Cotton Genetics 50R	0.97 g-m	873 hij	21.5 c-i	1.4 e-i
FiberMax 832	0.96 g-m	776 j	19.6 d-i	1.5 d-i
Americot 1532B2RF	0.95 g-m	1,020 f-i	25.4 a-g	1.1 i
DeltaPine 448B	0.95 g-m	1,114 efg	27.8 a-f	1.7 b-h
FiberMax 955LLB2	0.91 g-m	1,057 fgh	27.8 a-f	1.9 a-e
NexGen 3550RF	0.84 g-m	1,001 f-j	29.2 a-e	1.9 a-f
Deltapine 393	0.84 g-m	817 ij	25.0 a-g	1.6 c-i
All-Tex Atlas	0.78 h-m	1,119 efg	34.1 abc	1.5 d-i
FiberMax 958	0.78 i-m	1,191 def	35.9 ab	1.4 e-i
All-Tex Marathon B2RF	0.71 j-m	990 f-j	33.9 abc	1.3 ghi
Americot 427R	0.67 k-m	891 g-j	32.9 a-d	1.2 hi
Americot 8120	0.67 k-m	1,055 fgh	36.7 a	1.4 e-i
Cropland Genetics 4020B2RF	0.55 lm	873 hij	36.9 a	1.3 f-i
Beltwide Cotton Genetics 295	0.53 m	822 ij	36.4 a	1.5 e-i

[a]This measure is an attempt to combine both the wilt ratings and yield to determine the best varieties to grow in Verticillium wilt fields. The higher the numbers are the better varieties for Verticillium wilt fields. Relative wilt is the percent wilt, divided by the highest average wilt found in any variety. Relative yield is the yield divided by the highest average yield for any variety.
[b]Letters that are different indicate that the mean values were significantly different at P=0.05.

Discussion

The average relative wilt rating and relative yield ratings for varieties that were tested at a minimum of two sites during 2005 and/or 2006 were used to calculate the overall best varieties (Table 7). The highest rated variety was PM 2326RR, followed by FM 960BR, FM 989BR, FM 989B2R, and DP

455BR. The varieties with the highest average relative yields (on a 0 to 1 scale, Table 7) were: PM 2326RR (0.93), FM 960BR (0.92), DP 455BR (0.92), PhytoGen 480WR (0.91), FM 989B2R (0.89), and FM 989BR (0.89). The varieties with the lowest average relative yields were All-Tex Magnum RR (0.51), BCG 30R (0.55), BCG 50R (0.55), Cotton States 530001G (Cropland Genetics (CG) 3520B2RF, ST 4700B2RF, and Dyna-Gro (DG) 2242B2RF) (0.56), PhytoGen 310R (0.56), All-Tex Warrior RR (0.56), and BCG 28R (0.56). The varieties with the lowest average relative wilt values (Table 7)were: PM 2326RR (0.34), FM 989BR (0.47), FM 989B2R (0.47), FM 960B2R (0.48), and FM 966LL (0.49). The varieties with the highest average relative wilt ratings were: Americot 8120 (1.0), All-Tex Magnum RR (0.92), All-Tex Marathon B2RF (0.87), BCG 295 (0.83), and NexGen 3550RF (0.81).

Table 7. Relative ratings of varieties in terms of yield and wilt incidence, that were tested at a minimum of two sites during the 2005 and/or 2006 growing season.

Variety[a]	Av. Rel Yield[b]	Av. Rel. Wilt[c]	Best variety[d]	# of entries
Paymaster 2326RR	0.929	0.339	1.59	3
FiberMax 960BR	0.924	0.476	1.45	7
FiberMax 989BR	0.887	0.469	1.42	6
FiberMax 989B2R	0.890	0.471	1.42	4
Deltapine 455BR	0.921	0.517	1.40	4
FiberMax 958LL	0.857	0.502	1.36	2
FiberMax 966LL	0.805	0.489	1.32	2
Paymaster 2379RR	0.820	0.540	1.28	3
Deltapine 5690RR	0.840	0.570	1.27	2
FiberMax 960RR	0.794	0.538	1.26	7
Paymaster 2167R	0.885	0.635	1.25	3
NexGen 2448R	0.794	0.547	1.25	7
PhytoGen 470WR	0.830	0.590	1.24	2
FiberMax 989RR	0.840	0.608	1.23	5
FiberMax 960B2R	0.872	0.651	1.22	6
AFD 3602R	0.700	0.510	1.19	2
Deltapine 444BR	0.760	0.570	1.19	4
Stoneville 5242BR	0.774	0.588	1.19	3
FiberMax 5035LL	0.729	0.555	1.17	2
PhytoGen 480WR	0.913	0.748	1.17	2
FiberMax 981LL	0.751	0.588	1.16	2
Paymaster 2280BR	0.809	0.647	1.16	3
PhytoGen 125F	0.715	0.563	1.15	2
Stoneville 5303R	0.790	0.640	1.15	2
All-Tex Patriot RR	0.692	0.545	1.15	4
NexGen 1553R	0.789	0.646	1.14	3
Cotton States 370001G	0.654	0.529	1.13	3
Paymaster 2266RR	0.749	0.653	1.10	3
Cotton States 010001G	0.681	0.601	1.08	2
FiberMax 5045BR	0.760	0.680	1.08	3
Deltapine 424B2R	0.641	0.573	1.07	4
FiberMax 958	0.815	0.753	1.06	2

AFD 3511R	0.610	0.550	1.06	2
Stoneville 6636BR	0.730	0.670	1.06	2
Deltapine 449BR	0.760	0.700	1.06	2
All-Tex Atlas RR	0.636	0.582	1.05	4
PhytoGen 410R	0.610	0.570	1.04	2
Stoneville 4686R	0.620	0.600	1.02	2
Paymaster 2145R	0.740	0.720	1.02	2
Deltapine 434RR	0.580	0.590	0.99	5
Deltapine 555BR	0.720	0.730	0.99	2
NexGen 3969R	0.670	0.710	0.96	3
Stoneville 4575BR	0.750	0.790	0.96	2
Americot 262R	0.590	0.660	0.93	4
PhytoGen 310R	0.560	0.640	0.92	3
Deltapine 445BR	0.620	0.700	0.92	2
Cotton States 450001G	0.688	0.768	0.92	7
Deltapine 117B2RF	0.631	0.711	0.92	2
Deltapine 393	0.597	0.678	0.92	2
Deltapine 494RR	0.660	0.760	0.90	2
Stoneville 5599BR	0.590	0.700	0.89	2
Cotton States 530001G	0.559	0.670	0.89	4
All-Tex Warrior RR	0.560	0.700	0.86	2
Deltapine 432RR	0.630	0.770	0.86	2
NexGen 3550F	0.665	0.813	0.85	2
Beltwide Cotton Genetics 28R	0.560	0.710	0.85	4
Deltapine 5415RR	0.590	0.740	0.85	2
Americot 427R	0.632	0.782	0.85	2
Beltwide Cotton Genetics 50R	0.554	0.709	0.84	4
Beltwide Cotton Genetics 245	0.590	0.755	0.83	2
All-Tex Marathon B2F	0.687	0.866	0.82	2
Americot 821R	0.570	0.770	0.80	2
Beltwide Cotton Genetics 295	0.602	0.831	0.77	2
Beltwide Cotton Genetics 30R	0.540	0.780	0.76	2
Americot 8120	0.607	0.998	0.61	2
All-Tex Magnum RR	0.510	0.920	0.59	2

[a] Cotton States followed by a specific number represents multiple varieties, some of which are listed as the following: 010001G = Dyna-Gro (DG) 2215 B2RF, NexGen 3273 B2RF, BW 4021 B2RF, AM 1521 B2RF; 370001G = DG 2100 B2RF, Croplan Genetics (CG) 3020 B2RF, BW 3255 B2RF, AM 1504 B2RF; 450001G = DG 2520 B2RF, CG 4020 B2RF, BW 4630 B2RF, ST 4357 B2RF, AM 1532 B2RF; and 530001G = CG 3520 B2RF, ST 4700 B2RF, DG 2242 B2RF.

[b] Relative yield is the average yield of a variety divided by the average yield of the best yielding variety at that site.

[c] Relative wilt is the average incidence of wilt for a variety divided by the average incidence of wilt for the variety with the highest wilt rating at that site.

[d] The best variety was calculated by the formula: Relative yield + (1 – Relative wilt).

Progress of Greenhouse Screening

Field screening is preferred because susceptibility and tolerance are measured based on a disease rating (measure of symptom) but also production (yield). However, air temperatures are not conducive to disease development in all locations or years. A reliable greenhouse assay is also necessary to compensate for those years in which disease pressure is mild and resistance and susceptibility can't be determined in field trials. There are a number of methods for greenhouse screening including root-dip, stem and leaf inoculations and addition of microsclerotia to soil. The root dip method was the first tested. The general procedure was to grow the plants for approximately 6 wks, then soak the roots for one minute in a solution of spores. The plant is transplanted into soil and symptom development is rated. Ten isolates of *V. dahliae* were tested in a greenhouse assay using conidia at 10^6/ml of water on a susceptible (Beltwide Cotton Genetics [BCG] 24R) and partially resistant (FiberMax [FM] 989) variety. The procedure involved lightly wounding the roots and soaking in the conidial suspension for 3 min. Isolate DP1 gave the best results with wilt symptoms in 50% of the susceptible plants, and in none of the partially resistant plants. A second test was conducted to determine how long to soak the plants in the conidial suspension. BCG 24R and FM 989 were wounded by cutting roots more severely than the first test and soaked for 3, 10, or 45 minutes using isolate Mill1 at 10^6 conidia/ml. The largest difference between the susceptible and partially resistant varieties was found with soaking for 10 min. (BCG 24R = 63% with wilt, FM 989 = 17% with wilt). A third test was setup in the greenhouse to again examine the affect of 10 isolates of *V. dahliae*, while cutting the roots of BCG 24R and FM 989 severely, and then soaking for 10 min. at 10^6 conidia/ml. As a result of the three preliminary studies two isolates of *V. dahliae* were chosen to screen varieties. An initial variety screen was set up with 20 varieties including a susceptible and resistant check. The larger scale screening tests did not perform as well as when only a few varieties were used. Stem and leaf inoculation methods have been tested to no avail. Therefore, we decided to use the natural infection method. Microsclerotia were grown in large quantities, mixed in soil, and quantified. An experiment was set up using three fungal densities (0, 4, and 19 microsclerotia/cm^3 soil), and nine varieties (three susceptible, three intermediate, and three with partial resistance, based on field results), arranged in a randomized complete block design with 12 replications. The soil and microsclerotia are mixed in a cement mixer for 30 min. and then transferred to the appropriate pot. Seed (3/pot) were planted and then thinned to one plant per pot. The plants have currently been grown for 90 days. The wilt levels are fairly low at this time, with no treatment having > 16% plants with wilt symptoms. NG 3550RF (a susceptible variety) is showing a significant relationship between plant height and inoculum density, but the other varieties are not. The test will be continued for another month. A second test has been set up with three varieties (susceptible = ST 4554B2RF, intermediate = Cropland Genetics 3020B2RF, and resistant = PM 2326RR) and three inoculum levels (0, 150, and 450 microsclerotia/cm^3 soil) with 12 replications and two isolates of *V. dahliae*. We are looking for a protocol that can distinguish between the resistant and susceptible varieties with either wilt symptoms (preferable) or height differences, and do it within 90 days after planting. The biggest difficulty with the microsclerotia approach is getting a good mix of the microsclerotia in soil. We are in the process of purchasing a high quality blending system from Patterson-Kelly that should take care of this problem. The benefits of the microsclerotia system is that the infection process is natural and won't bypass any resistance mechanisms; we will have good control over the precise amount of inoculum present that each plant is exposed to; and finally, there is no skill necessary to inoculate plants, any person can do equally well by mixing the soil and inoculum for the same amount of time.

Objective 2: *Integrate Black Root Rot resistance into Upland cotton* - Black root rot of cotton, incited by the soilborne fungus *Thielaviopsis basicola*, can cause substantial yield loss and reduced fiber quality. In the present study, we have shown a strong genetic component that controls resistant and susceptible reactions resulting from the *Thielaviopsis* infestation. Upland cottons were highly susceptible to the disease but two diploid species, *G. arboreum* var. "PI 1415" and *G. herbaceum* var. "A20", did show varying levels of resistance. Line "PI 1415" had some level of resistance to *T. basicola* when challenged with relatively low levels of inoculum (Wheeler, et al., 1999, Plant Disease 83:831-833), but was completely susceptible when challenged with high levels of the fungus (500 to

1,000 spores/cm^3 soil). A high level of resistance was identified in "A20". Plants exhibited less than 5% root necrosis and the few observable disease lesions were a series of small points rather than a concentrated area of the root.

Our initial experiment using DNA marker technology to identify and map genes that confer resistance to the fungus is ongoing. A mapping population was created between the highly resistant or immune parent "A20" and the less resistant parent "PI 1415". Parent and progeny (F_2) plants were grown in soil infested with *Thielaviopsis basicola* for 6-7 wks at 19^oC. The seedlings were scored for root necrosis and transplanted into the greenhouse approximately 7-10 days after emergence. DNA was isolated from each individual and fingerprinted using DNA marker technology (SSR and RFLP). Marker loci alignment (mapping) and the analysis of both qualitative and quantitative resistant phenotypes is near completion. We are progeny testing seed from F_2 individuals to minimize the number of disease escapes that can occur [i.e. 2 of 10 PI 1415 (susceptible parent) plants were classified as resistant] and improve the overall reliability of the disease ratings.

We are currently in the position to begin the transfer of resistance gene(s) into Upland cotton. The technical feasibility to transfer important genes from diploid cotton into Upland was established by R.L. Knight in the 1940's. Knight successfully transferred bacterial blight resistance genes from diploid *G. arboreum* species into Upland cottons. This B_6 gene has historically been a very important source of resistance to the most virulent *Xcm* races, including Race 18. Many modern cotton breeding programs around the world are using the B_6 gene to minimize a potential bacterial blight epidemic.

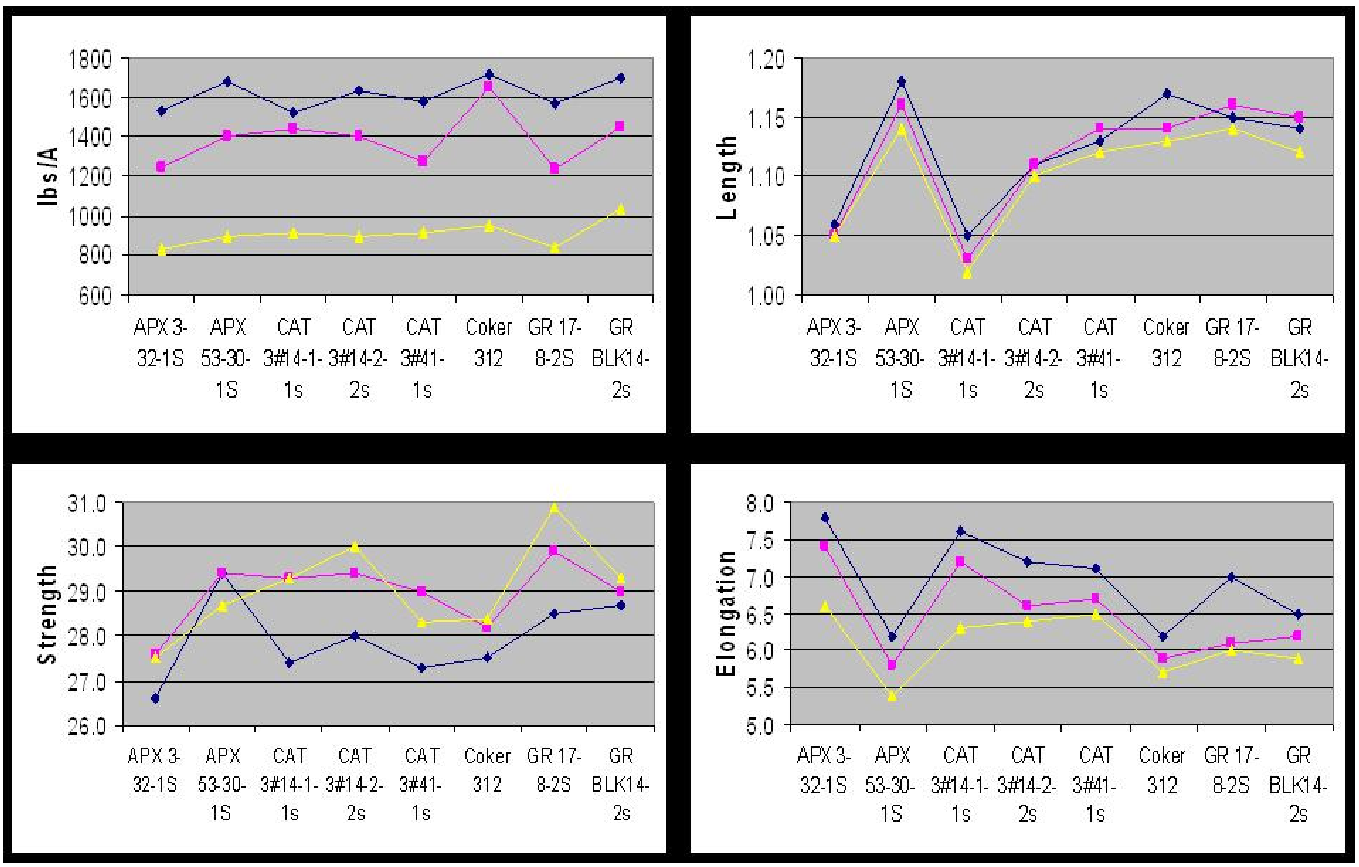
We intend to transfer the gene(s) conferring resistance to Black Root Rot using those methods initially developed by Knight. DNA markers will aid in this process by improving both selection efficiency and shorten the development time-line of Upland cotton resistant to *T. basicola*. The specific steps we propose are (1) cross resistant and susceptible diploid progenitor species, (2) double the chromosome complement of F_1 individuals, (3) identify and select synthetic tetraploid progeny, and (4) cross the synthetic tetraploid with elite Upland varieties. It would be unduly optimistic to assume all steps will be completed within the funding period; however, we expect to complete Steps 1 and 2. We have made a number of crosses between our resistant A20 and *G. hirsutum* and three plants, one of which is mature were recovered. The mature plant (F1) is being used to produce selfed seed and as both a male and female parent in crosses. Most of the crosses with it as a female have been successful. We recently discovered five *G. hirsutum* lines with resistance to *T. basicola* in another project and have planted seed from each. Four of the lines were grown to maturity (one would not germinate) and have been crossed with conventional varieties. We will be screening populations in the greenhouse for *T. basicola* resistance and then transplanting resistant progeny to the field in 2008. This material may represent a way to get black root rot resistance into useful germplasm much more rapidly than from the *G. herbaceum* line.

Objective 3: *Assay cotton germplasm for superior tolerance to abiotic stress* – Advancements in biotechnology have clearly changed the seed industry and the many traits offered to the cotton grower. Technologies that improve a plants ability to tolerate conditions in which water becomes unavailable (drought tolerance) will be the next generation of traits offered to the growers. The WTCG has focused on developing practical methods to accurately and confidently evaluate these technologies prior to commercialization. To asses the usefulness of our methods we are evaluating three in-house technologies (genes) involved in the reduction of reactive oxygen intermediates (ROI's). Increased production of ROI's that occurs in stressed plants during photosynthesis, respiration, and other metabolic processes and genes that reduce these ROIs may improve abiotic (drought or salinity) tolerance. Three such transgenic technologies that express catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) have been developed by our group. The efficacy of these technologies is being evaluated in field, greenhouse, and laboratory experiments. We have also intercrossed these lines to stack drought tolerant genes and eventual test the synergetic effect that may result. The specific steps of this project are as follows:

1. Evaluate lines containing the CAT, APX, and GR transgenes in Texas Tech's variable irrigation nursery, and
2. Develop genetic lines with enhanced tolerance to drought and salt stress.

Progress of Transgenic Testing

We are currently using a split-block design to evaluate 8 lines (2 APX, 3 CAT, 2 GR, and 1 non-transgenic) based on three watering treatments. Each line was planted in 2 row plots, 15 meters in length that are replicated four times within each irrigation block. This experimental design was successfully employed during the 2005 and 2006 seasons to generate yield, HVI, and AFIS data. Analyses of variance is being used to compare genotypes within each PET zone and regression stability analyses used to determine lint yield and fiber quality stability across the three PET zones. Fiber production and quality was determined by harvesting each plot, determining seed cotton weight, calculating lint percentages from ginned samples, and HVI and AFIS testing fiber samples. Adverse seasonal conditions have affected the stand (plant population) in this year's trial. Consequently, we expect to see higher CV's for yield and a reduced confident in this assessment. Fiber quality characters are currently being evaluated by the International Textile Center in Lubbock Texas. Although preliminary the performance of a few fiber traits are enhance when compare to non-transgenic Coker 312. A few transgenes (APX 32-1S and CAT 3#14-1-1s) appear to negatively affect fiber length.



Each transgene has beneficial properties yet the additive relationship may be greater than the sum of each transgenes alone. Therefore, different gene combinations (APX x CAT, APX x GR, and CAT x GR) are being developed to test in a future TSS proposal. In this current phase of the study we will develop genetic tools and lines to enable the stacking of these different transgenes into single cotton lines. Efficient introgression of these transgenes from the host variety Coker 312 into breeding lines requires a robust molecular assay to quickly determine the presence and zygosity of the transgene insert. These assays will provide the ability to identify homozygous progeny with one simple PCR reaction. To accomplish this goal, selected transgenic lines were grown in the greenhouse and tissue samples were harvested and processed for DNA isolation. Molecular analysis of transgenic cotton lines is underway and identification of transgene inserts has been accomplished. Flanking genomic sequences of each transgenic line have been cloned and sequenced using Genomic RACE methods using specific primers designed to anneal in the T-DNA insert and ligated adapter sequences to provide an anchor within the flanking genomic sequence. This sequence information is now being used for the design multiplex PCR assays that give unequivocal results regarding transgene copy number. While such an assay may not be necessary for single transgene introgression, it is required for simultaneous selection for homozygosity in lines with multiple transgene inserts. In addition, sequencing of the transgene insert will allow for identification of specific insertion events.

These PCR-based zygosity assays will provide substantial improvements in the effectiveness of transgene introgression and make the task of moving these transgenes to adapted lines much more efficient. To use these assays, selected transgenic lines and "stacked" lines, containing two or more transgenes, will be field-tested during the 2007 growing season. These plants will be grown under a differential irrigation regime and evaluated for yield and fiber quality. Transgene data from these plants will provide for direct correlation between transgene inheritance and phenotype.

Progress of Drought and Salt Tolerance Testing

Obsolete cotton race stocks and wild cotton accessions from the various collections around the world are being screened for traits that will be useful in developing drought and salt resistant cottons. Commercial cotton cultivars in use today have become progressively more inbred, and many workers believe that they lack the genetic diversity for strong change. By contrast, the genetic bases of the obsolete cotton race stocks and wild cottons are broad and are much more likely to be a source of useful, natural traits.

The collection of *Gossypium hirsutum* race stock accessions maintained at the Southern Crops Research Lab, USDA at College Station, Texas is a source of genetic diversity that can be used to provide useful, heritable traits for breeding programs. One phase of plant growth that needs to be characterized for these accessions is seedling growth, including root development. Information gained by seedling growth studies could be utilized in breeding work aimed toward coping with adverse environments including drought.

Studies to develop seedling data on these accessions are being conducted at the Crops Genetic Research Facility at the Texas A&M Research and Extension Center at Lubbock, Texas.

Materials and Methods

Cotton accessions were grown in 30 inch tubes made from 3 inch PVC pipe. Each tube was split lengthwise. The two halves were held together by hose clamps while plants were growing. The clamps were removed at harvest and one side of the tube removed to give easy access to the root system for washing. Granular diatomaceous earth was used as growing medium. This lightweight material is easily washed from roots and can be reused. Drainage was allowed. Adequate water and fertilizer were applied to obtain maximum growth rates. Plants were grown for 20 days with day temperatures maintained at 75-85°F and night at 67-69°F. After washing, taproot and shoot length were measured and plants were separated into roots and shoots, and dried at 100°F for 48 hours. Parameters obtained were: taproot length, shoot length, total root and shoot weights, and shoot to root ratio. All experiments were conducted using a RCB design with five blocks. TX020 was used as a control in all seedling tests so separate tests can be compared. Twenty-two experiments were conducted in 2006, testing a total of 154 accessions.

Results and Discussion

Results from four growth studies conducted during 2006 are presented in Tables 1-4. Significant differences were found for all parameters. However, shoot to root rations were significantly different only in test 29 (Table 2). There were wide ranges in values between accessions, especially for weight. Accession TX0307 (Table 4) had significantly larger values for all length and weight measurements than other entries except TX0020 in test 31. Each weight value for TX0307 was over twice those of TX0313. In test 28, root length of TX0279 was only 56% as long as TX0280 (Table 1).

Some accessions emphasized shoot growth (weight) compared to root growth while others exhibited the opposite response as indicated by the shoot to root ratios. In test 29 (Table 2), TX0284 and TX0282 produced three times as much shoot weight compared to root weight, while shoot weight for TX0292 and TX0020 was only approximately twice the root weight.

The accessions studied exhibited a wide range in expression of seedling growth characteristics, thus providing breeders with material that could fit their needs for useful traits regardless of their application.

Table 1. Growth test number 28 in tubes, Lubbock, Texas, 2006.[a]

	Length (cm)		Dry weight (mg)			Shoot to
Accession	Root	Shoot	Root	Leaf+shoot	Plant total	root ratio
TX0280	53.1 a	7.8 ab	0.091 ab	0.197 ab	0.288 abc	2.2 a
TX0267	52.6 ab	7.9 ab	0.087 abc	0.221 a	0.308 ab	2.6 a
TX0277	48.8 abc	6.0 c	0.073 bcd	0.168 bc	0.241 cd	2.3 a
TX0268	43.2 bcd	5.8 c	0.062 d	0.149 c	0.212 d	2.4 a
TX0278	41.3cd	6.7 bc	0.073 bcd	0.180 abc	0.253 bcd	2.5 a
TX0020	40.9 cd	8.3 a	0.110 a	0.217 a	0.327 a	2.0 a
TX0275	36.8de	6.2 c	0.063 cd	0.141 c	0.205 d	2.2 a
TX0279	30.2e	6.1 c	0.065 cd	0.147 c	0.212 d	2.5 a

[a]Means within a column, followed by the same letter, are not significantly different (P>0.05, LSD).

Table 2. Growth test number 29 in tubes, Lubbock, Texas, 2006.[a]

	Length (cm)		Dry weight (mg)			Shoot to
Accession	Root	Shoot	Root	Leaf+shoot	Plant total	root ratio
TX0292	44.6 a	7.5 ab	0.079 ab	0.169 a	0.248 ab	2.1 cd
TX0281	41.7 ab	7.8 ab	0.075 ab	0.172 a	0.247 ab	2.3 bcd
TX0020	40.1 ab	7.5 ab	0.092 a	0.192 a	0.284 a	2.1 d
TX0284	37.5 abc	6.7 bc	0.055 cd	0.147 a	0.202 bc	3.0 ab
TX0285	34.4 abc	5.6 c	0.067 bc	0.157 a	0.224 bc	2.3 bcd
TX0289	32.3 bc	5.7 c	0.047 d	0.125 a	0.172 c	2.8 abc
TX0282	29.6 c	6.0 c	0.048 d	0.153 a	0.202 bc	3.2 a
TX0286	28.2 c	8.1 a	0.065 bcd	0.165 a	0.230 ab	2.5 abcd

[a]Means within a column, followed by the same letter, are not significantly different (P>0.05, LSD).

Table 3. Growth test number 30 in tubes, Lubbock, Texas, 2006.[a]

	Length (cm)		Dry weight (mg)			Shoot to
Accession	Root	Shoot	Root	Leaf+shoot	Plant total	root ratio
TX0295	35.2 a	8.0 b	0.057 bcd	0.133 bc	0.190 c	2.4 a
TX0297	32.7 ab	5.9 d	0.058 bcd	0.131 cd	0.189 cd	2.3 a
TX0020	31.9 abc	7.9 b	0.084 a	0.175 a	0.260 a	2.1 a
TX0301	30.3 bc	9.0 a	0.068 b	0.153 b	0.221 b	2.2 a
TX0298	29.5 bc	7.3 bc	0.054 cd	0.114 cde	0.168 cde	2.1 a
TX0303	28.5bc	5.9 d	0.042 e	0.097 e	0.138 e	2.3 a
TX0304	27.6 c	5.9 d	0.060 bc	0.119 cd	0.179 cd	2.0 a
TX0296	27.4 c	7.1 c	0.047 de	0.111 de	0.159 de	2.5 a

[a]Means within a column, followed by the same letter, are not significantly different (P>0.05, LSD).

Table 4. Growth test number 31 in tubes, Lubbock, Texas, 2006.[a]

	Length (cm)		Dry weight (mg)			Shoot to
Accession	Root	Shoot	Root	Leaf+shoot	Plant total	root ratio
TX0307	35.9 a	8.6 a	0.105 a	0.232 a	0.337 a	2.2 a
TX0020	30.9 ab	6.4 b	0.090 a	0.194 b	0.284 b	2.2 a
TX0306	26.9 bc	5.4 c	0.057 bc	0.144 cd	0.201 cd	2.7 a
TX0308	25.8 bc	5.4 c	0.070 b	0.158 cd	0.228 c	2.3 a
TX0311	25.6 bc	4.8 c	0.063 bc	0.175 bc	0.238 bc	2.8 a
TX0305	24.2 c	5.2 c	0.048 c	0.127 de	0.175 b	2.8 a
TX0310	23.0 c	5.5 c	0.052 bc	0.142 cd	0.193 cd	2.9 a
TX0313	21.8 c	5.2 c	0.045 c	0.105 e	0.150 d	2.4 a

[a]Means within a column, followed by the same letter, are not significantly different (P>0.05, LSD).

Objective 4: ***Create cotton lines with enhanced fiber quality –*** The short fiber length of cotton varieties grown across the state of Texas has historically reduced the price received in both domestic and international markets. The future of the Texas cotton industry is dependent upon the development of commercial varieties which produce superior fiber quality and commercially competitive lint yields. There is an increased emphasis on growing cotton varieties whose fiber meets or exceeds the minimal standards of the export market. To be considered as "Export Class A" a bale must have a stable length 35/32 (1.09 inches) or greater to avoid substantial discounts in foreign markets. The average staple length for 2003 and 2004 Texas High Plains cotton was 1.06 and 1.07 inches, respectively.

Progress of Enhancing Fiber Quality

Crosses were made between superior quality mutants developed by TexasTech University with two Acala lines (1517-99 and NM24016), two *G. barbadense* lines (89590x and 8810x) and a line from the

University of Georgia (94042x). In 2005 ($F_{3:4}$), 113 individual plants from these crosses were selected based on agronomic appearances and fiber quality and planted as $F_{4:5}$ in 2006 in two replicates at Lubbock, TX along with six check varieties. Fifteen mutant x Acala selections, twelve mutant x *G. barbadense* selections and one mutant x University of Georgia line selection gave fiber lengths ranging from 1.30 to 1.37 inches which were significantly higher than that of the check varieties. FiberMax 958 had fibers 1.17 inches long and FiberMax 989 had fibers that were 1.22 inches long. Thirty crosses had fiber strengths ranging from 34–37 g/tex. Both FiberMax varieties had fibers that were 30 g/tex strong.. The majority of the crosses exhibited micronaire readings within the acceptablr range of 3.9 to 4.2. Fiber length uniformity for most of the crosses were excellent. More than 60% of the crosses had fiber length uniformity of 85 to 88%. The corresponding values for FM 958 and FM 989 were 85% and 86%, respectively. AFIS analyses during 2005 and 2006 confirmed the superiority of some of these crosses to the two FiberMax varieties. This was demonstrated by lower nep size and nep count, very high upper quartile lengths (up to 1.48), lower short fiber content by weight and number, higher fineness values, lower immature fiber content and higher maturity ratios. In summary, this project has identified lines with exceptional fiber quality for the short season production environment of West Texas. The lint yield data for these crosses and check varieties will be available later on when the materials are ginned.

Extensive fiber and yarn evaluations of 2005 and 2006 fiber from 18 mutant lines have identified mutants that are homozygous for the partially naked seed trait (up to 48% nakedness). These lines have significantly increased seed oil content and improved delinting efficiency. They also had significantly reduced seed coat neps and short fiber contents, improved yarn quality and AFIS fiber maturity. Another aspect of the merit of these lines is that they have fast and low energy requirements for oil extraction and ginning.