

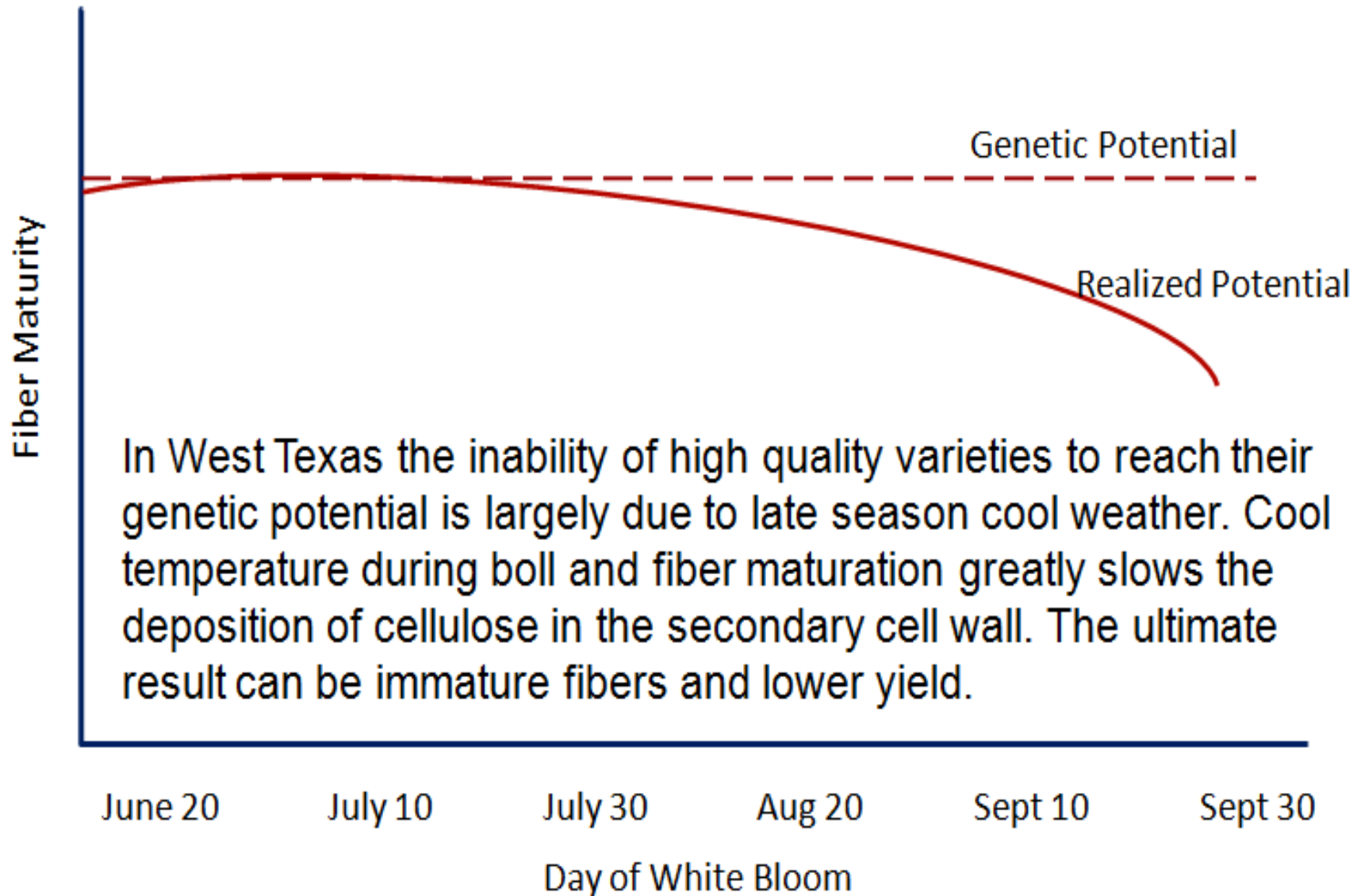


**OVER-EXPRESSED
ALTERNATIVE OXIDASE
GENE EFFICACY ON
FIBER GROWTH AND
MATURATION IN COTTON**

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Fiber maturity



Annual Micronaire Quality Report from the Lubbock Classing Office

Micronaire	2007	2008	2009	2010	2011
24 & Below	0.1	2.1	3.0	*	0.1
25-26	0.3	4.4	3.9	0.2	0.4
27-29	1.6	10.4	8.8	1.2	1.2
30-32	4.6	13.4	11.8	4.0	2.7
33-34	6.1	10.0	9.4	5.6	3.3
35-36	9.0	10.7	10.7	9.1	4.7
37-42	41.0	32.0	37.2	45.9	30.8
43-49	34.7	16.1	14.9	32.7	51.9
50-52	2.4	0.8	0.3	1.0	4.5
53 & Above	0.3	0.1	*	0.1	0.4
34 & Below	12.7	40.3	36.9	11.0	7.7
Average	4.1	3.6	3.7	4.0	4.3

Rationale of Research

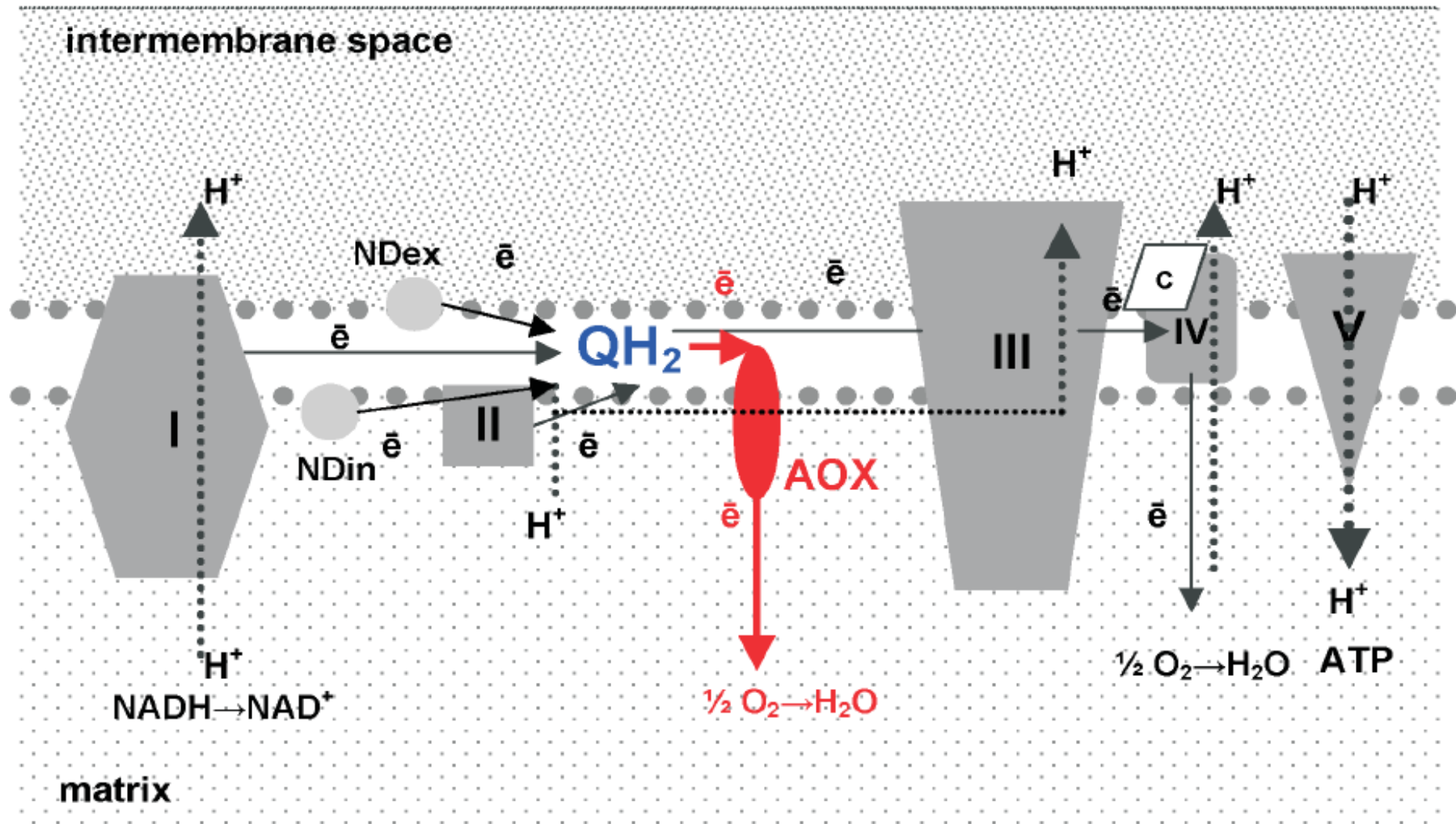
The inflorescence of the voodoo lily will rise to 10°C above ambient temperatures as a result of a shift from the normal oxidative respiration to an alternative oxidative pathway.



**Thermogenic plant
has heat sink tissue**

Skunk cabbage- *Lysichiton americanum*

AOX in plant mitochondrial respiratory chain



Alternative oxidase in plant mitochondrial respiratory chain.

Enhanced Expression of AOX

Objective

- Examine the phenotypic consequences of enhanced AOX expression in cotton exposed to cool temperatures
 - initial growth and development
 - boll development and maturation

Hypothesis

- Cotton plants with enhanced expression of the *AOX* gene will maintain consistent fiber quality characteristics (HVI and AFIS) under suboptimal late season cool temperatures.

Gene Efficacy Experiments

Growth Chamber Experiment

- Two transgenic events (66-6 and 94-20), null (94-3), and Coker 312-17
- 12 replications (RCBD)
- Two temperature cycles (28/22°C and 22/15°C)
- Assess gene efficacy on growth measures

AOX Efficacy

Plant growth at 28/22°C day/night temperature

Genotype	Plant height (cm)	Number of nodes	Avg. internode length (cm)	Plht to 2nd node (cm)	Intnode length to 2nd node (cm)	Plht from 2nd node (cm)	Avg Internode from 2nd node (cm)	Shoot fresh wt (gm)	Leaf area (cm ²)
T 94-20	40.1 ^b	8.0	4.2 ^b	14.7	8.5	25.5 ^b	4.2 ^b	54 ^a	1273 ^a
T 66-6	43.7 ^a	7.7	5.0 ^a	15.9	9.9	27.9 ^a	4.9 ^a	56 ^a	1295 ^a
Null 94-3	38.0 ^b	7.6	4.3 ^b	14.4	8.7	23.6 ^b	4.3 ^b	49 ^b	1189 ^b
C-312	40.0 ^b	7.5	4.6 ^b	15.1	9.2	25.0 ^b	4.5 ^b	49 ^b	1127 ^b
LSD _(0.05)	2.30	ns	0.36	not analyzed	not analyzed	1.95	0.34	4.1	74

Plant growth at 22/15°C day/night temperature

Genotype	Plant height (cm)	Number of nodes	Avg. internode length (cm)	Plht to 2nd node (cm)	Intnode length to 2nd node (cm)	Plht from 2nd node (cm)	Avg Internode from 2nd node (cm)	Shoot fresh wt (gm)	Leaf area (cm ²)
T 94-20	23.5 ^a	5.7	2.7 ^a	15.3 ^a	7.1 ^a	8.6 ^a	3.3 ^a	34.7 ^a	658 ^a
T 66-6	19.9 ^b	5.3	2.2 ^b	13.8 ^c	5.9 ^c	5.9 ^b	2.4 ^b	34.6 ^a	649 ^{ab}
Null 94-3	20.6 ^b	5.4	2.4 ^b	14.0 ^{bc}	6.7 ^{ab}	6.8 ^b	2.5 ^b	32.4 ^b	624 ^{bc}
C-312	21.4 ^b	5.5	2.3 ^b	14.9 ^{ab}	6.3 ^{bc}	6.7 ^b	2.5 ^b	32.9 ^b	607 ^c
LSD _(0.05)	1.57	ns	0.24	0.94	0.58	1.26	0.41	1.48	29

Gene Efficacy Experiments

Field Experiment

- 2010, 2011, and 2012
- Texas Tech Research Farm, Lubbock TX
- Two transgenic events (66-6 and 94-20), null (94-3), and Coker 312-17
- Two planting dates (April and May)
- 13M plots (single row)
- 3 replications (RCBD)
- A time-course analysis of fiber development was conducted using day of white bloom

Time-course Tagging of Fiber Development



- ✚ Daily (Mon-Fri) all white blooms were tagged
- ✚ Each week a different tag color was used to track weekly boll development
- ✚ All bolls were harvested by the week of white bloom

✚ A tenth sample (pooled) represents all untagged bolls that developed from Saturday and Sunday white blooms

✚ HVI and AFIS tests for fiber characteristics

Week	Tag colors
1	White
2	Yellow
3	Pink
4	Red
5	Green
6	Orange
7	Light blue
8	Yellow with pink
9	White with pink

Micronaire 2010

Table 1. Mean fiber micronaire trait of April 2010 Entries

Genotype	Week of White Bloom									
	1	2	3	4	5	6	7	8	9	pooled†
C312	4.60	4.55	5.22	5.40	5.23	5.00	4.88	4.31	3.87	5.11
Null	5.06	5.30	5.49	5.60	5.46	5.06	4.79	4.60	4.93	5.27
66-6	5.36	5.43	5.98	5.80	5.77	5.45	5.34	4.27	NA‡	5.14
94-20	5.11	5.03	5.50	5.65	5.39	4.96	4.56	3.72	NA	4.91
LSD _{0.05}	0.32	0.26	0.13	0.18	0.17	0.25	0.35	NS*	NA	NS

† Pooled represents all untagged bolls that developed from Saturday and Sunday w hite blooms.

‡ NA, not available.

*NS, not significant.

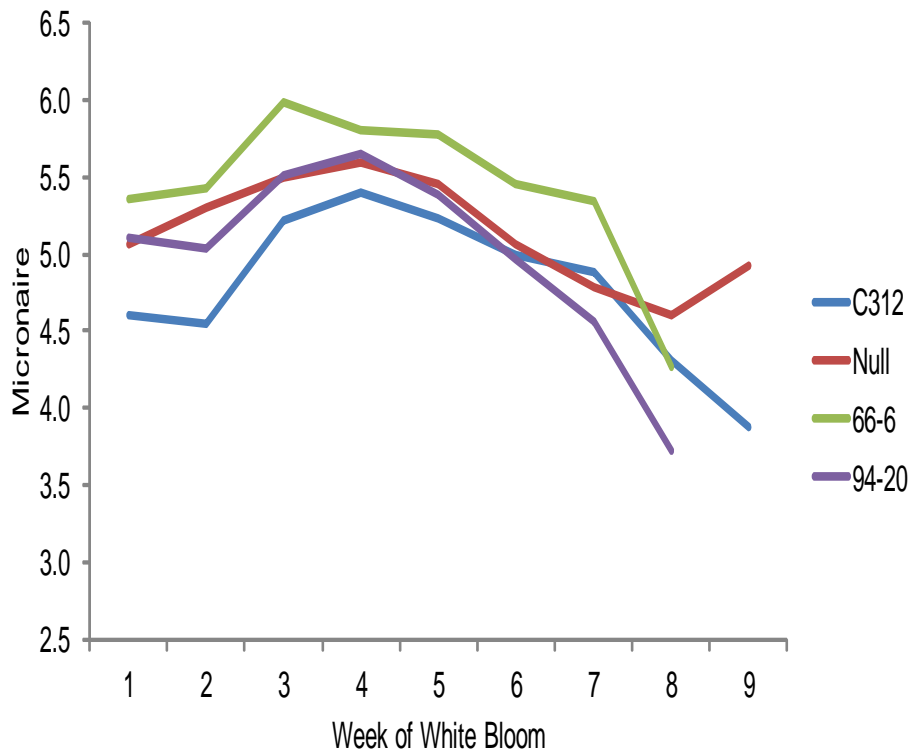


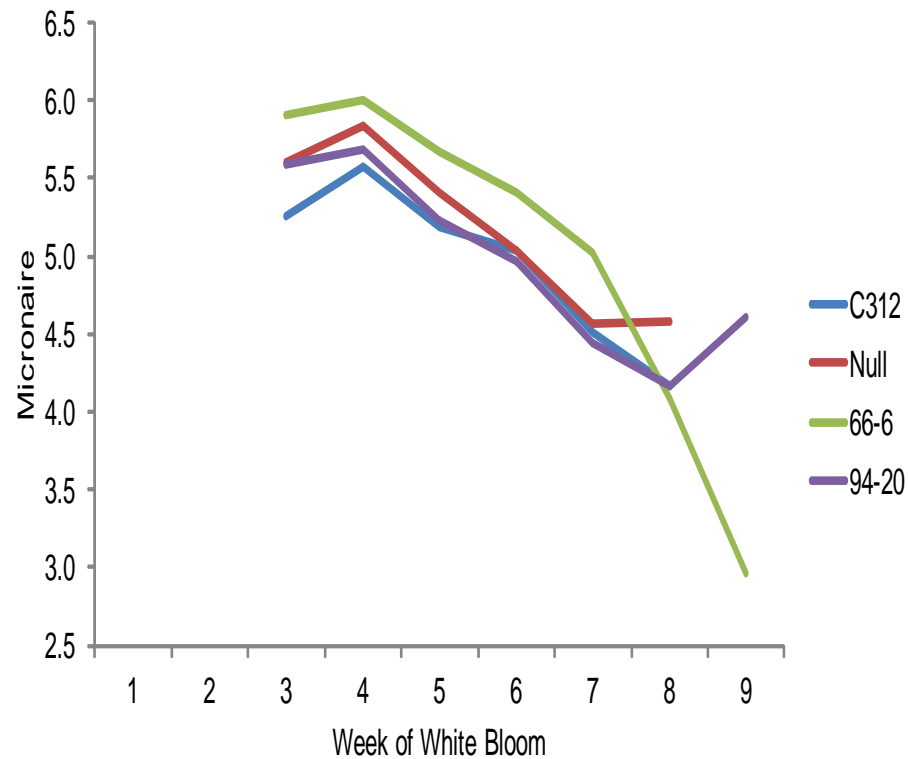
Table 1. Mean fiber micronaire trait of May 2010 Entries

Genotype	Week of White Bloom									
	1	2	3	4	5	6	7	8	9	pooled†
C312	NA‡	NA	5.25	5.57	5.18	5.03	4.51	4.17	NA	5.07
Null	NA	NA	5.60	5.84	5.41	5.04	4.57	4.58	NA	5.04
66-6	NA	NA	5.90	6.01	5.68	5.41	5.02	4.08	2.97	5.27
94-20	NA	NA	5.59	5.69	5.23	4.96	4.44	4.16	4.61	4.97
LSD _{0.05}	NA	NA	0.17	0.15	0.25	0.17	0.22	NS*	NA	NS

† Pooled represents all untagged bolls that developed from Saturday and Sunday w hite blooms.

‡ NA, not available.

*NS, not significant.



Micronaire 2011

Table 1. Mean fiber micronaire trait of April 2011 Entries

Genotype	Week of White Bloom									
	1	2	3	4	5	6	7	8	9	pooled†
C312	5.01	5.03	5.34	4.85	4.80	4.63	5.28	NA‡	NA	4.65
Null	5.47	5.39	5.56	5.21	5.02	NA	NA	NA	NA	5.33
66-6	5.71	5.77	5.94	5.64	5.19	5.40	NA	NA	NA	5.65
94-20	5.33	5.32	5.47	5.02	4.88	4.90	NA	NA	NA	5.10
LSD _{0.05}	0.21	0.16	0.17	0.20	NS*	NA	NA	NA	NA	NS

† Pooled represents all untagged bolls that developed from Saturday and Sunday white blooms.

‡ NA, not available.

*NS, not significant.

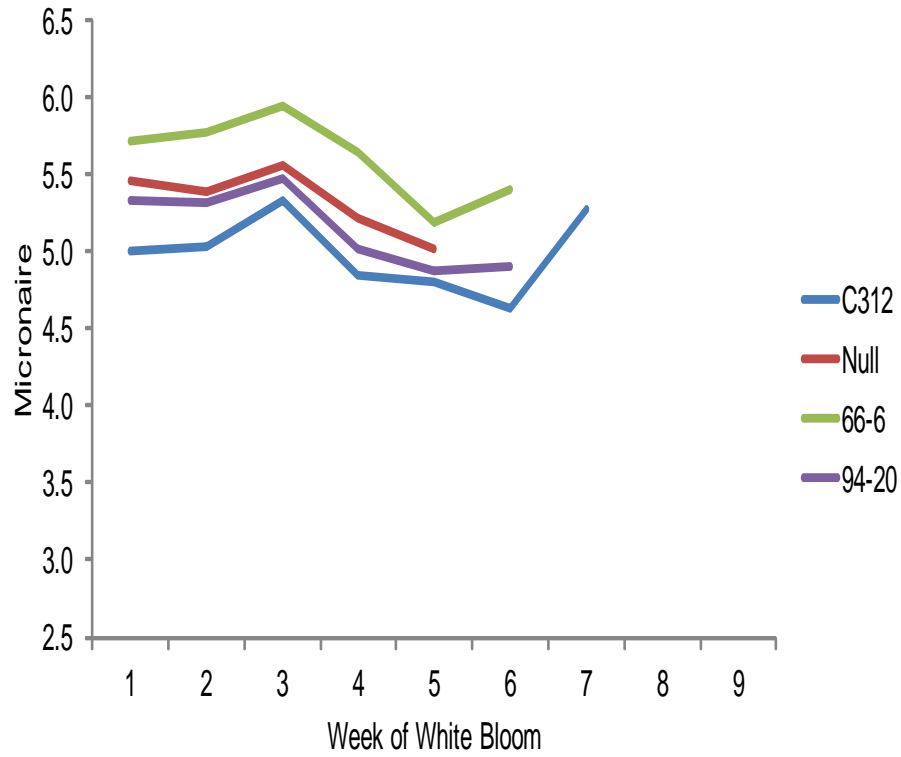


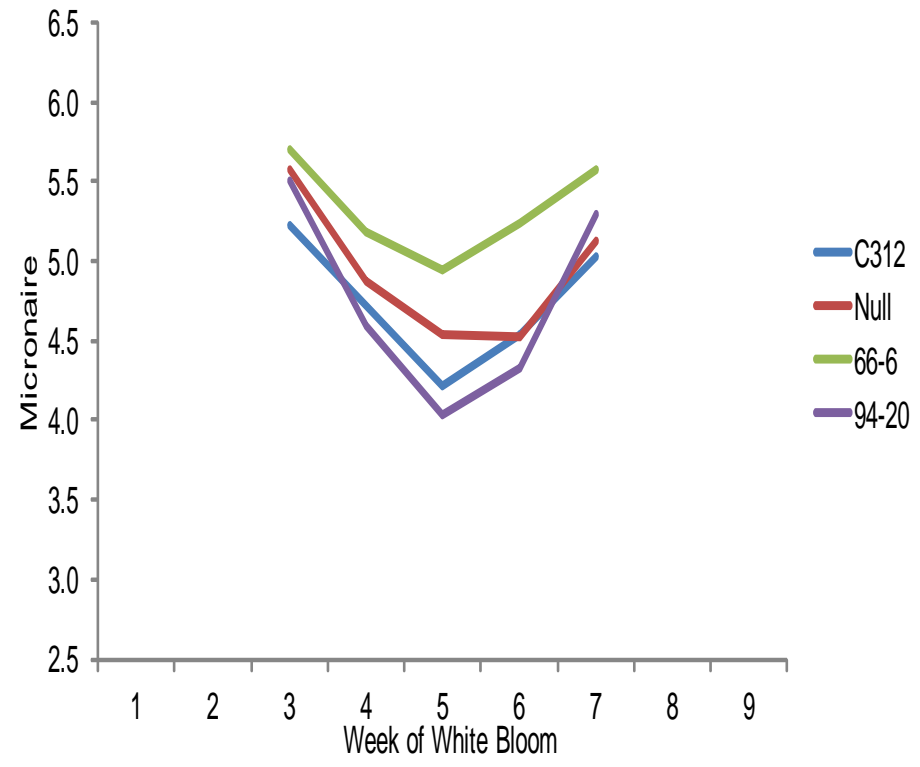
Table 1. Mean fiber micronaire trait of May 2011 Entries

Genotype	Week of White Bloom									
	1	2	3	4	5	6	7	8	9	pooled†
C312	NA†	NA	5.22	4.72	4.22	4.54	5.03	NA	NA	4.86
Null	NA	NA	5.58	4.87	4.54	4.52	5.13	NA	NA	5.13
66-6	NA	NA	5.70	5.18	4.95	5.24	5.57	NA	NA	5.34
94-20	NA	NA	5.51	4.59	4.04	4.33	5.30	NA	NA	5.01
LSD _{0.05}	NA	NA	0.25	0.24	0.36	0.27	NS*	NA	NA	0.16

† Pooled represents all untagged bolls that developed from Saturday and Sunday white blooms.

‡ NA, not available.

*NS, not significant.



Fineness 2010

Table 1. Mean fiber finess trait of April 2010 Entries

Genotype	Week of White Bloom									pooled [†]
	1	2	3	4	5	6	7	8	9	
C312	170.0	165.7	190.0	207.3	197.7	195.0	184.3	173.3	169.3	191.7
Null	192.0	189.7	201.0	212.0	204.0	193.3	182.3	183.7	177.5	198.7
66-6	202.0	195.7	215.0	215.7	214.7	204.0	197.3	173.0	193.3	203.0
94-20	191.0	190.7	195.0	212.7	200.3	194.7	177.3	160.3	172.3	187.7
LSD _{0.05}	9.9	5.2	5.9	NS*	5.3	NS	NS	NS	NS	NS

[†] Pooled represents all untagged bolls that developed from Saturday and Sunday w hite blooms.

*NS, not significant.

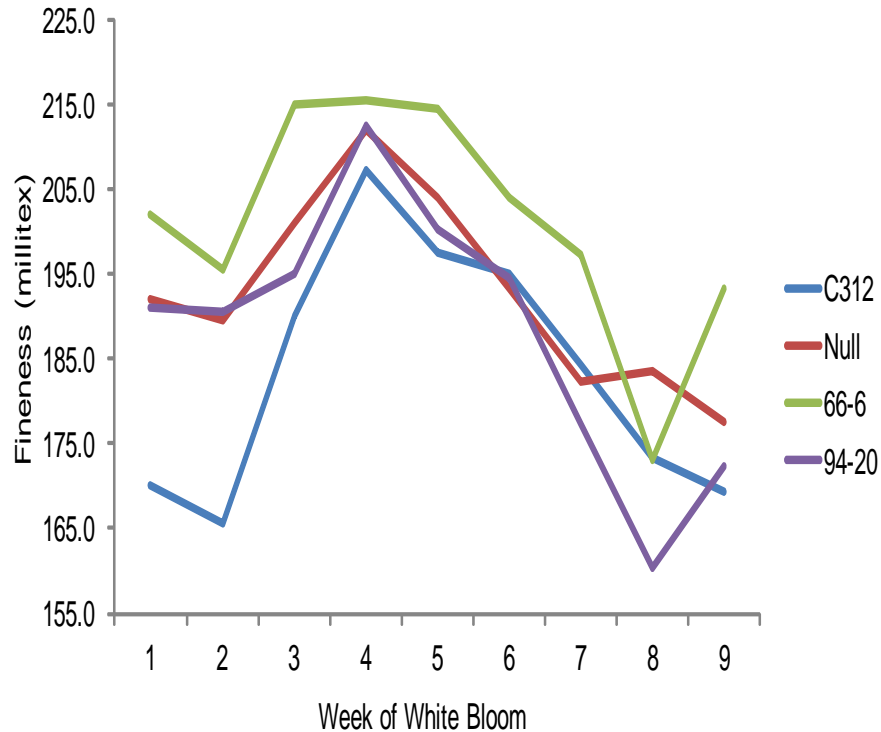


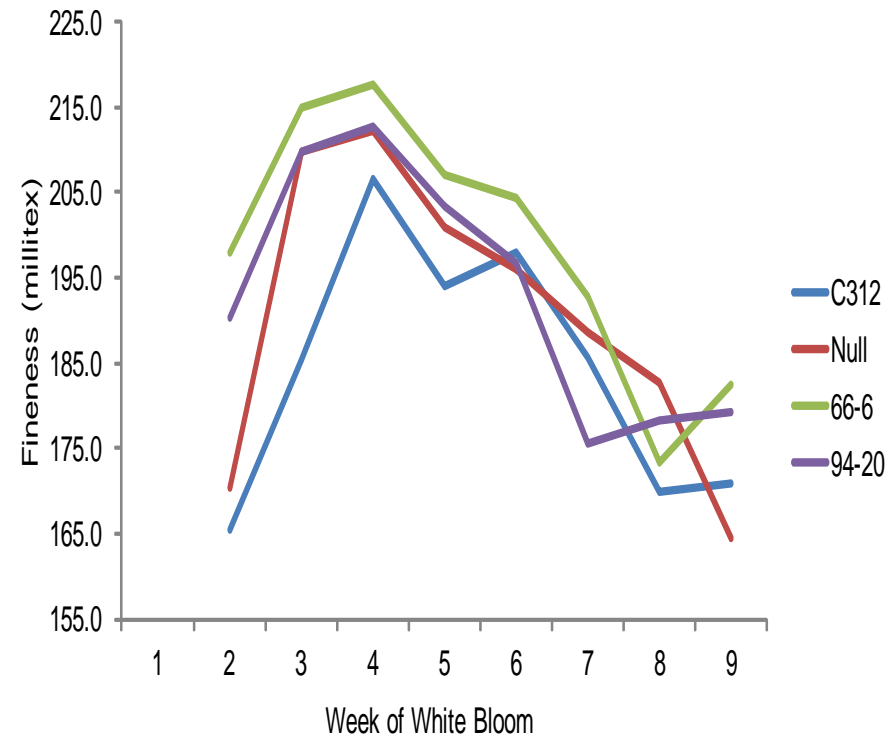
Table 1. Mean fiber finess trait of May 2010 Entries

Genotype	Week of White Bloom									pooled [†]
	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	
C312	NA [‡]	165.5	185.3	206.7	194.0	198.0	185.7	170.0	171.0	191.0
Null	NA	170.5	209.7	212.3	201.0	196.0	188.7	182.7	164.5	189.3
66-6	NA	198.0	215.0	217.7	207.0	204.3	192.7	173.3	182.5	197.0
94-20	NA	190.3	209.7	212.7	203.3	196.7	175.7	178.3	179.3	190.0
LSD _{0.05}	NA	NS*	5.6	NS	3.4	NS	NS	NS	NS	NS

[†] Pooled represents all untagged bolls that developed from Saturday and Sunday w hite blooms.

[‡] NA, not available.

*NS, not significant.



Fineness 2011

Table 1. Mean fiber fineness trait of April 2011 Entries

Genotype	Week of White Bloom									
	1	2	3	4	5	6	7	8	9	pooled [†]
C312	187.7	186.7	194.7	183.7	181.0	165.7	195.7	188.3	183.5	180.0
Null	199.3	199.3	204.0	193.7	187.3	192.3	205.0	171.5	NA [‡]	192.3
66-6	205.7	211.7	215.0	211.7	195.7	199.3	217.0	210.0	204.0	201.3
94-20	198.7	198.3	203.0	190.0	184.7	186.5	209.3	217.0	179.0	188.7
LSD _{0.05}	7.5	7.5	8.0	8.2	NS [*]	NS	NS	NS	NS	8.8

[†] Pooled represents all untagged bolls that developed from Saturday and Sunday w hite blooms.

[‡] NA, not available.

^{*}NS, not significant.

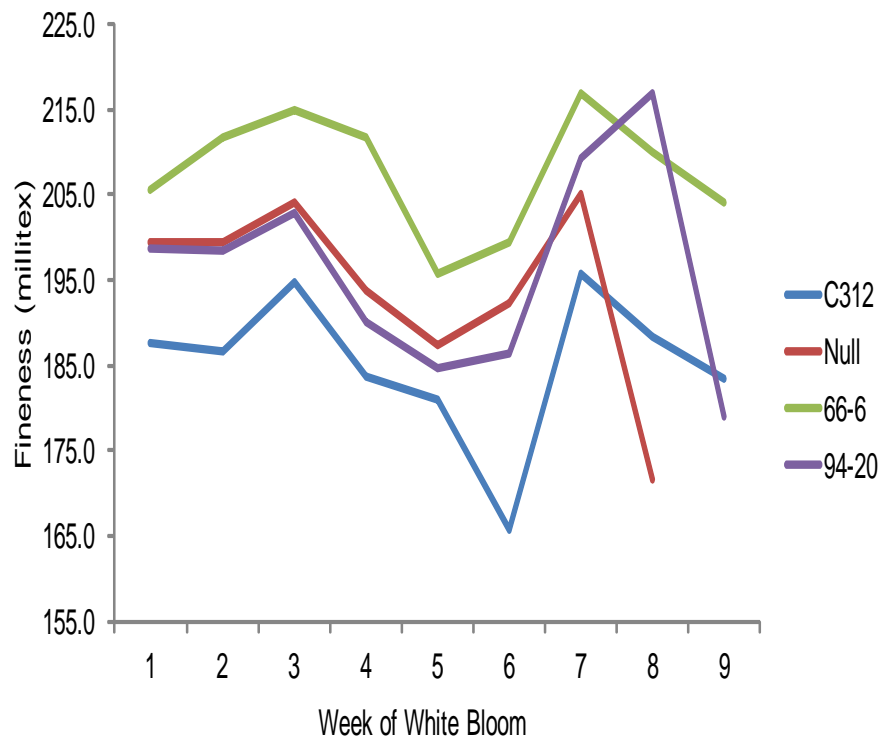


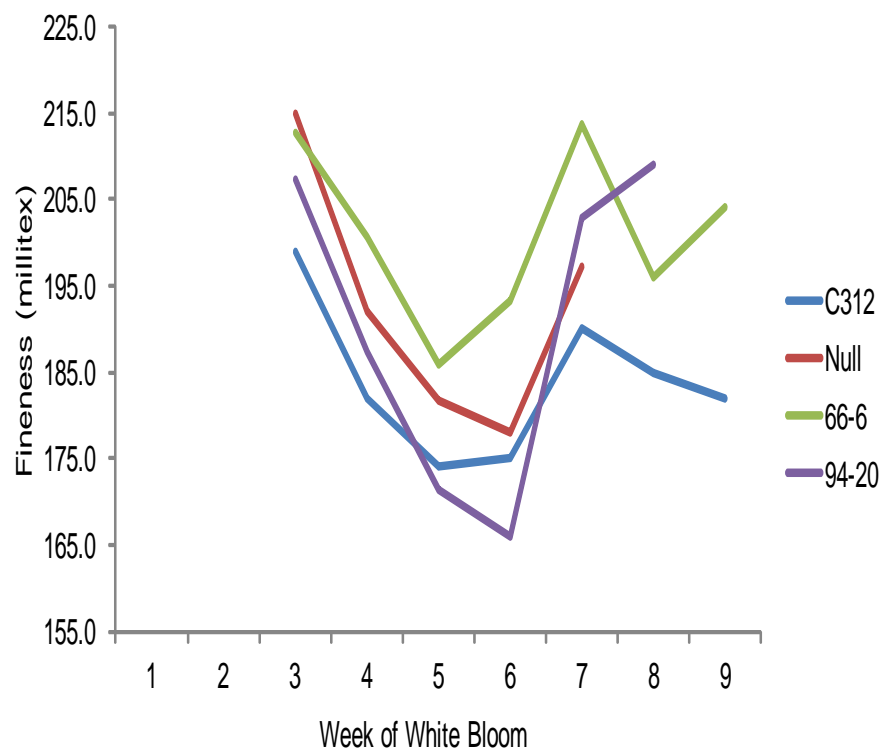
Table 1. Mean fiber fineness trait of May 2011 Entries

Genotype	Week of White Bloom									
	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	pooled [†]
C312	NA [‡]	NA	199.0	182.0	174.0	175.0	190.0	185.0	182.0	184.3
Null	NA	NA	215.0	192.0	181.7	178.0	197.3	NA	NA	192.7
66-6	NA	NA	212.7	200.7	186.0	193.3	213.7	196.0	204.0	198.0
94-20	NA	NA	207.3	187.3	171.3	166.0	203.0	209.0	NA	190.3
LSD _{0.05}	NA	NA	7.4	7.8	7.1	8.5	NS [*]	NA	NS	5.6

[†] Pooled represents all untagged bolls that developed from Saturday and Sunday w hite blooms.

[‡] NA, not available.

^{*}NS, not significant.



Maturity Ratio 2010

Table 1. Mean maturity ratio trait of April 2010 Entries

Genotype	Week of White Bloom									pooled [†]
	1	2	3	4	5	6	7	8	9	
C312	0.92	0.91	0.95	0.99	0.98	0.98	0.95	0.92	0.91	0.97
Null	0.95	0.94	0.96	0.99	0.97	0.96	0.93	0.93	0.91	0.97
66-6	0.99	0.97	1.00	1.00	1.01	0.97	0.96	0.89	0.95	0.99
94-20	0.95	0.95	0.96	1.00	0.97	0.96	0.91	0.87	0.89	0.94
LSD _{0.05}	0.03	0.02	0.01	NS*	0.02	0.01	NS	NS	NS	0.02

[†] Pooled represents all untagged bolls that developed from Saturday and Sunday white blooms.

*NS, not significant.

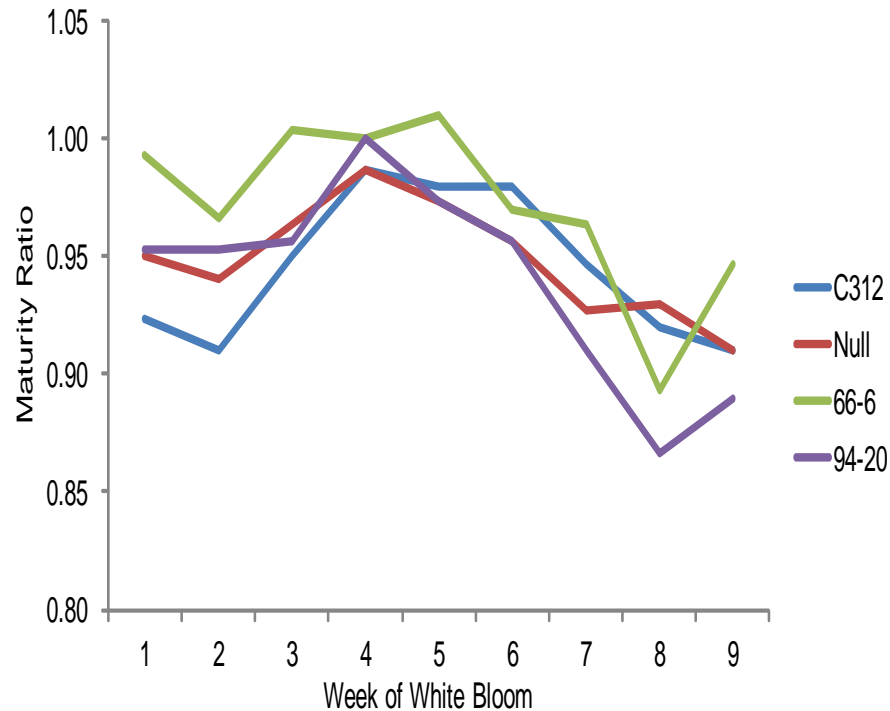


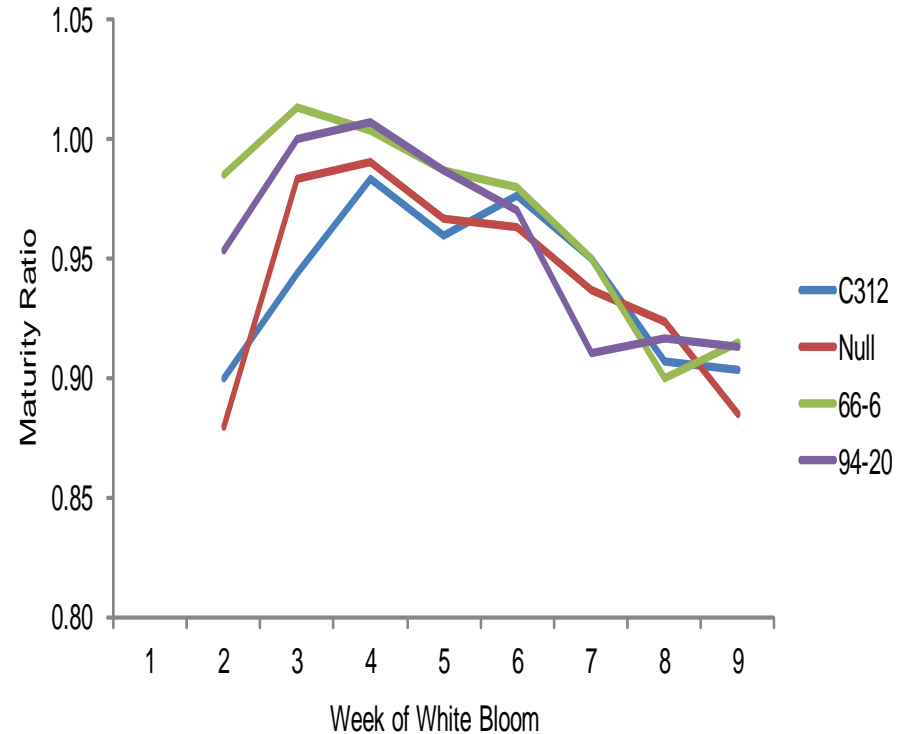
Table 1. Mean maturity ratio trait of May 2010 Entries

Genotype	Week of White Bloom									pooled [†]
	1	2	3	4	5	6	7	8	9	
C312	NA [‡]	0.90	0.94	0.98	0.96	0.98	0.95	0.91	0.90	0.95
Null	NA	0.88	0.98	0.99	0.97	0.96	0.94	0.92	0.89	0.93
66-6	NA	0.99	1.01	1.00	0.99	0.98	0.95	0.90	0.92	0.96
94-20	NA	0.95	1.00	1.01	0.99	0.97	0.91	0.92	0.91	0.95
LSD _{0.05}	NA	NS*	0.02	NS	0.01	NS	0.02	NS	NS	NS

[†] Pooled represents all untagged bolls that developed from Saturday and Sunday white blooms.

[‡] NA, not available.

*NS, not significant.



Maturity Ratio 2011

Table 1. Mean maturity ratio trait of April 2011 Entries

Genotype	Week of White Bloom									
	1	2	3	4	5	6	7	8	9	pooled [†]
C312	0.98	0.98	0.99	0.96	0.96	0.94	1.01	0.98	0.99	0.94
Null	0.98	0.99	0.99	0.97	0.96	0.98	1.01	0.95	NA [‡]	0.96
66-6	1.01	1.02	1.02	1.00	0.98	0.98	1.03	1.03	1.01	0.98
94-20	0.99	0.99	0.99	0.97	0.95	0.96	1.01	1.02	0.95	0.96
LSD _{0.05}	NS [*]	0.02	0.02	0.02	NS	NS	NS	NS	NA	NS

[†] Pooled represents all untagged bolls that developed from Saturday and Sunday w white blooms.

[‡] NA, not available.

^{*}NS, not significant.

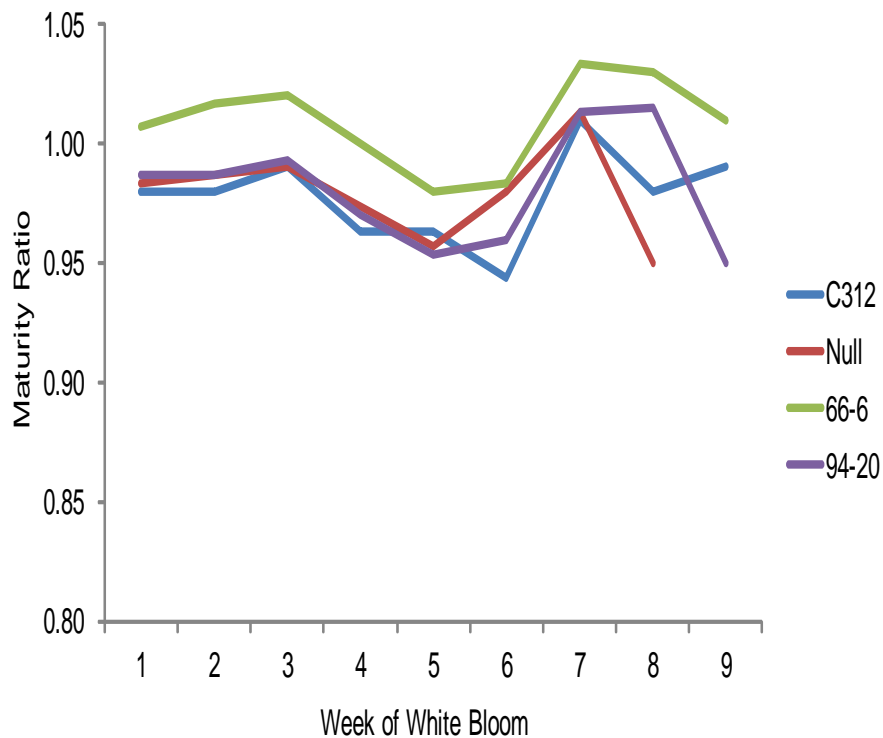


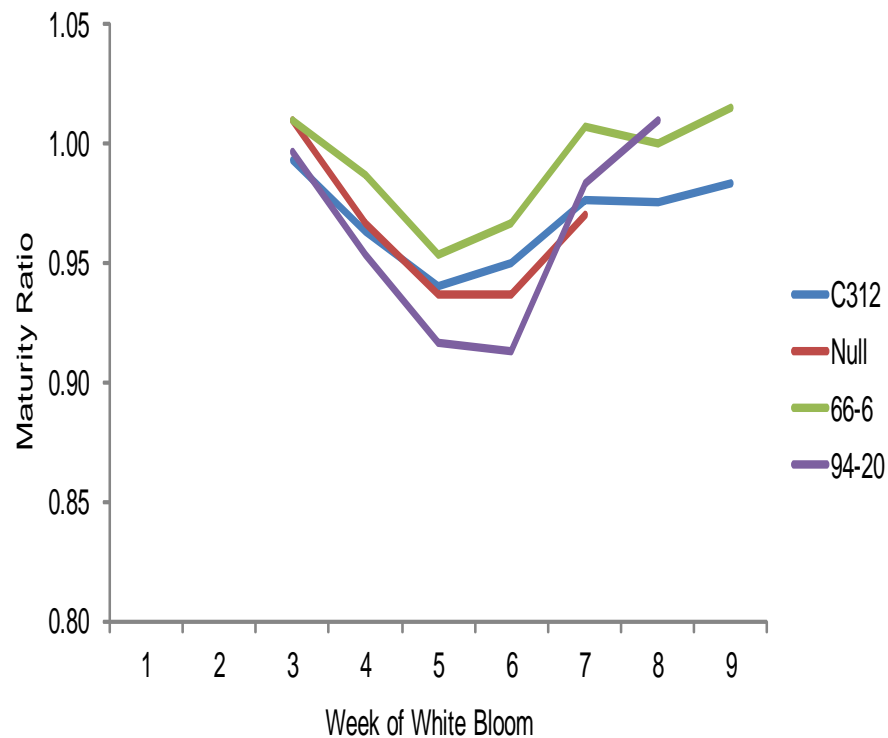
Table 1. Mean maturity ratio trait of May 2011 Entries

Genotype	Week of White Bloom									
	1	2	3	4	5	6	7	8	9	pooled [†]
C312	NA [‡]	NA	0.99	0.96	0.94	0.95	0.98	0.98	0.98	0.96
Null	NA	NA	1.01	0.97	0.94	0.94	0.97	NA	NA	0.96
66-6	NA	NA	1.01	0.99	0.95	0.97	1.01	1.00	1.02	0.98
94-20	NA	NA	1.00	0.95	0.92	0.91	0.98	1.01	NA	0.96
LSD _{0.05}	NA	NA	NS [*]	NS	NS	NS	NS	NA	NA	NS

[†] Pooled represents all untagged bolls that developed from Saturday and Sunday w white blooms.

[‡] NA, not available.

^{*}NS, not significant.



Conclusions

- ✦ The growth chamber experiments suggest that AOX plants with enhanced expression have conferred improved seedling growth under cool temperatures.
- ✦ The average seasonal temperatures during the months of August to October were above average in 2010 and 2011.
- ✦ The percentage of low MIC bales (≤ 3.4) classified in 2010 and 2011 were 11.0 and 7.7%. This compared to 40.3 and 36.9% in 2008 and 2009 (Lubbock Classing Office Final Quality Report).

Conclusions

✚ Although an insufficient level of temperature stress was seen in 2010 and 2011 to appropriately test our hypothesis, there was a clear trend to suggest that AOX may have altered 66-6 for micronaire, fineness, and maturity when compared to the other genotypes tested.

✚ The partitioning of boll development using a multicolor tagging of the white blooms has been a valuable technique to investigate the time-course crop development of fiber characteristics.

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