CURRENT PERFORMANCE OF VIP TRAITS IN COTTON

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PAST AND CURRENT BT COTTON TECHNOLOGIES

Company	1st generation (single gene)	2nd generation (dual gene)	3rd generation (multi-gene)	3rd generation (2017)
Bayer	Bollgard (Cry1Ac)	Bollgard 2 (Cry1Ac+Cry2Ab)		Bollgard 3 (Cry1Ac+Cry2Ab+Vip3A)
Corteva		WideStrike (Cry1Ac+Cry1F)	WideStrike 3 (Cry1Ac+Cry1F+Vip3A)	
BASF		TwinLink (Cry1Ab+Cry2Ae)		TwinLink Plus (Cry1Ab+Cry2Ae+Vip3A)
Homogeny across crops				
Crop	**Cry1A**	**Cry1F**	**Cry2**	**Vip3A**
Cotton	Cry1Ac, Cry1Ab	Cry1F	Cry2Ab, Cry2Ae	Vip3A
Corn	Cry1Ab	Cry1F	Cry2Ab2	Vip3A
	Cry1A.105 (Cry1Ab, Cry1Ac, Cry1F)			
Both crops	**Cry1As, Cry1F, Cry2As and Vip3A**			

SURVEY BIOASSAY PROCEDURE

- Bollworms/corn earworm collected from the field as larvae
- Overnight delivery to lab in College Station
- Reared to F1 or F2 generation and then bioassays
- Tested for response to Cry1Ac, Cry2Ab2, Cry1F and Vip3A
- Diet overlay bioassays
  - Test 6-8 Bt concentrations and a control
  - Used 16-32 neonate larvae, replicated 4 times for each concentration; allowed to feed for 7 days
- Record number alive/dead, instar and weight of survivors
- Compare field populations to a standard laboratory strain (Benzon)
  - Dead = Actual dead larvae + 1st instar larvae
  - Dose response bioassay: Probit analysis for LC50 and their 95% CL.
  - Resistance ratio = LC50 of a field population / LC50 of the susceptible strain.

Insect strain	LC_{50} (µg/cm^2)	Resistance ratio
CBW-BZ-SS-TAMU	0.11	1.0
CBW-TX-SS-TAMU	0.09	1.2
CBW-AR-LE-VT2P	>31.60	>316.00
CBW-AR-TR-VT2P	>31.60	>316.00
CBW-LA-AA-VT2P	8.97	81.55
CBW-LA-AA-BG2	>31.60	>316.00
CBW-TN-JN-VT2P	>31.60	>316.00
CBW-BZ-SS-USDA	0.18	1.0
CBW-PK-AR-NBt corn	>31.60	>316.00
CBW-HB-LA-CC	12.30	68.33

Cry1Ac Resistance Survey Results

- Arkansas
- Louisiana
- Tennessee

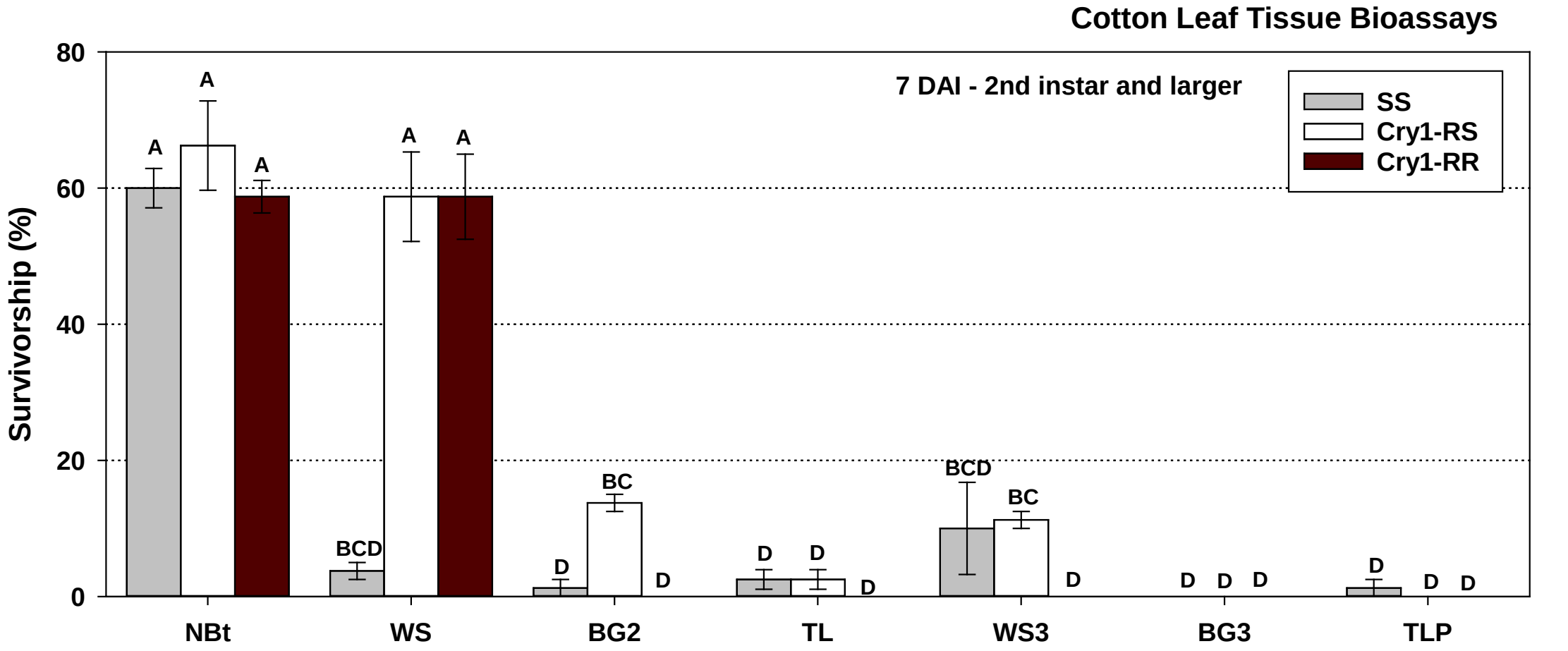
Insect strain	LC_{50} (µg/cm^2)	Resistance ratio
CBW-BZ-SS-TAMU	0.11	1.0
CBW-TX-SS-TAMU	0.09	1.2
CBW-MS-SE-Leptra	2.37	21.55
CBW-MS-SE-VT2P	>31.60	>316.00
CBW-MS-SK-VT2P	>31.60	>316.00
CBW-BZ-SS-USDA	0.18	1.0
CBW-CA-MS-CC	>31.60	>175.56
CBW-CO-MS-CC	>31.60	>175.56
CBW-FA-MS-Bt corn	14.26	77.8
CBW-GD-MS-CC	>31.60	>175.56
CBW-MK-MS-CC	>31.60	>175.56
CBW-MB-MS-NBt corn	>31.60	>175.56
CBW-NZ-MS-CC	>31.60	>175.56
CBW-OB-MS-CC	>31.60	>175.56
CBW-RF-MS-Bt corn	>31.60	>175.56
CBW-SV-MS-NBt corn	>31.60	>175.56
CBW-WA-MS-CC	>31.60	>175.56
CBW-TL-MS-Bt cotton-F2	6.23	34.01

Cry1Ac Resistance Survey Results

- Mississippi

Mid-South
100% with RRs > 10X

COTTON TECHNOLOGY EFFICACY TO CRY1Ac-SS, RS & RR GENOTYPES



*Also resistant to Cry1F

Insect strain	LC_{50} (µg/cm^2)	Resistance ratio
CBW-BZ-SS-TAMU	0.63	1.0
CBW-TX-SS-TAMU	0.70	1.1
CBW-AR-LE-VT2P	4.42	7.01
CBW-AR-TR-VT2P	8.06	12.79
CBW-LA-AA-VT2P	15.15	24.05
CBW-LA-AA-BG2	>20.00	>31.75
CBW-LA-WB-VT2P	1.62	2.57
CBW-TN-JN-VT2P	>20.00	>31.75
CBW-BZ-SS-USDA	0.98	1.0
CBW-PK-AR-NBt corn	3.16	3.16
CBW-HB-LA-CC	>10.00	>10.20

Cry2Ab2 Resistance Survey Results

- Arkansas
- Louisiana
- Tennessee

Insect strain	LC_{50} (µg/cm^2)	Resistance ratio
CBW-BZ-SS-TAMU	0.63	1.0
CBW-TX-SS-TAMU	0.70	1.1
CBW-MS-SE-Leptra	2.05	3.25
CBW-MS-SE-VT2P	>20.00	>31.75
CBW-MS-SK-VT2P	>20.00	>31.75
CBW-BZ-SS-USDA	0.98	1.0
CBW-CA-MS-CC	>10.0	>10.20
CBW-CO-MS-CC	>10.0	>10.20
CBW-FA-MS-Bt corn	>10.0	>10.20
CBW-GD-MS-CC	>10.0	>10.20
CBW-MK-MS-CC	>10.0	>10.20
CBW-MB-MS-NBt corn	>10.0	>10.20
CBW-NZ-MS-CC	>10.0	>10.20
CBW-OB-MS-CC	>10.0	>10.20
CBW-RF-MS-Bt corn	>10.0	>10.20
CBW-SV-MS-NBt corn	>10.0	>10.20
CBW-WA-MS-CC	>10.0	>10.20
CBW-TL-MS-Bt cotton-F2	1.86	1.88

Cry2Ab2 Resistance Survey Results

- Mississippi

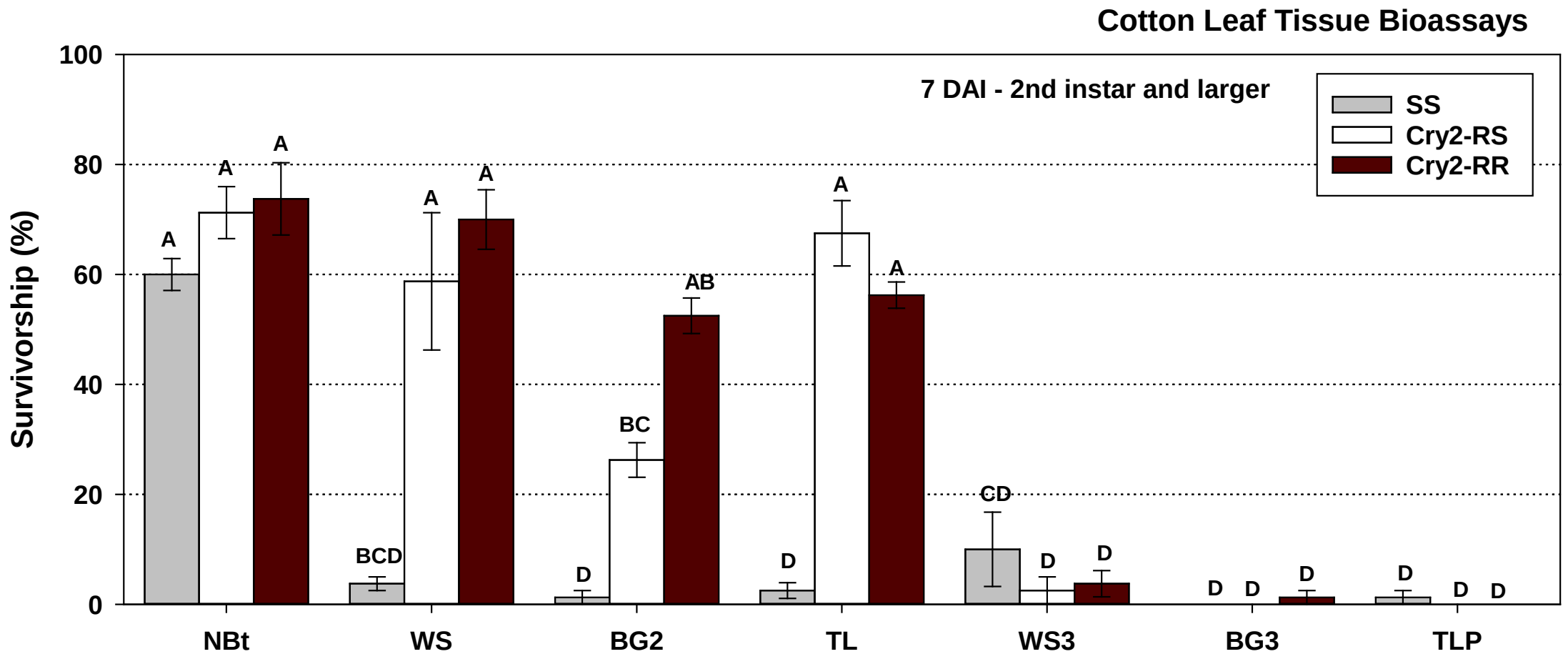
Mid-South
78.26% with RRs > 10X

CRY2Ab2 RESISTANCE INHERITANCE

Population	Genotype	N	LC_{50} (95% CI) (µg/cm²)	Slope ± SE	X^2	df	Resistance Ratio
CBW-TX-SS	SS	512	0.70 (0.51,0.67)	1.53 ± 0.15	40.2	26	--
CBW-G13-Cry2Ab2-RR	RR	512	286.35 (65.44, 5243)	0.41± 0.07	32.6	26	409.1
F1a: RR♂*SS♀	RS	512	14.96 (6.06, 105.08)	1.26 ± 0.37	43.3	26	21.4
F1b: RR♀*SS♂	RS	512	15.73 (8.88, 37.33)	1.18 ± 0.20	33.7	26	22.5
Pooled F1	RS	1024	15.37 (9.48, 30.19)	1.20 ± 0.18	79.6	54	22.0

- Cry2Ab2 RR resistance is very high
- Resistance is dominant (based on >10X resistance ratio of RS)
- However, this resistance is no complete and may be classified as partially recessive (because resistance ratio of RS is skewed towards susceptibility)
- Other backcrossing data suggest that there may be more than one gene involved and that the other gene(s) may be sexually linked

COTTON TECHNOLOGY EFFICACY TO CRY2A-SS, RS & RR GENOTYPES



*Also resistant to Cry1F & Cry1Ac

Insect strain	LC_{50} (µg/cm^2)	Resistance ratio-BZ	Resistance ratio-TX
CBW-BZ-SS-TAMU	0.69	1.0	4.05
CBW-TX-SS-TAMU	0.17	-0.25	1.0
CBW-AR-LE-VT2P	0.39	-1.80	4.06
CBW-AR-TR-VT2P	0.15	-4.60	-1.13
CBW-LA-AA-VT2P	0.23	-3.00	1.35
CBW-LA-AA-BG2	0.24	-2.88	1.41
CBW-LA-WB-VT2P	0.14	-4.93	-1.21
CBW-TN-JN-VT2P	0.32	-2.16	1.88
CBW-BZ-SS-USDA	0.39	1.0	
CBW-PK-AR-NBt corn	0.21	-1.86	

Vip3Aa39 Resistance Survey Results

- Arkansas
- Louisiana
- Tennessee

Insect strain	LC_{50} (µg/cm^2)	Resistance ratio-BZ	Resistance ratio-TX
CBW-BZ-SS-TAMU	0.69	1.0	4.05
CBW-TX-SS-TAMU	0.17	-4.05	1.0
CBW-MS-SE-Leptra	2.21	3.20	13.00
CBW-MS-SE-VT2P	0.08	-8.63	-2.13
CBW-MS-SK-VT2P	0.16	-4.31	-1.06
CBW-BZ-SS-USDA	0.39	1.0	
CBW-FA-MS-Bt corn	0.22	-1.77	
CBW-GD-MS-CC	0.09	-4.33	
CBW-MB-MS-NBt corn	0.14	-2.79	
CBW-MK-MS-CC	0.16	-2.44	
CBW-OB-MS-CC	0.12	-3.25	
CBW-RF-MS-Bt corn	0.16	-2.44	
CBW-SV-MS-NBt corn	0.32	-1.22	
CBW-WA-MS-CC	0.15	-2.60	
CBW-TL-MS-Bt cotton-F2	0.04	-0.12	

Vip3Aa39 Resistance Survey Results

- Mississippi

Mid-South
0.00% with RRs > 10X

- Failures in Vip cotton are the exception, not the rule
- Most of the time occurs under very high pressure
  - Trap crop scenarios
- Bloom and bloom tags

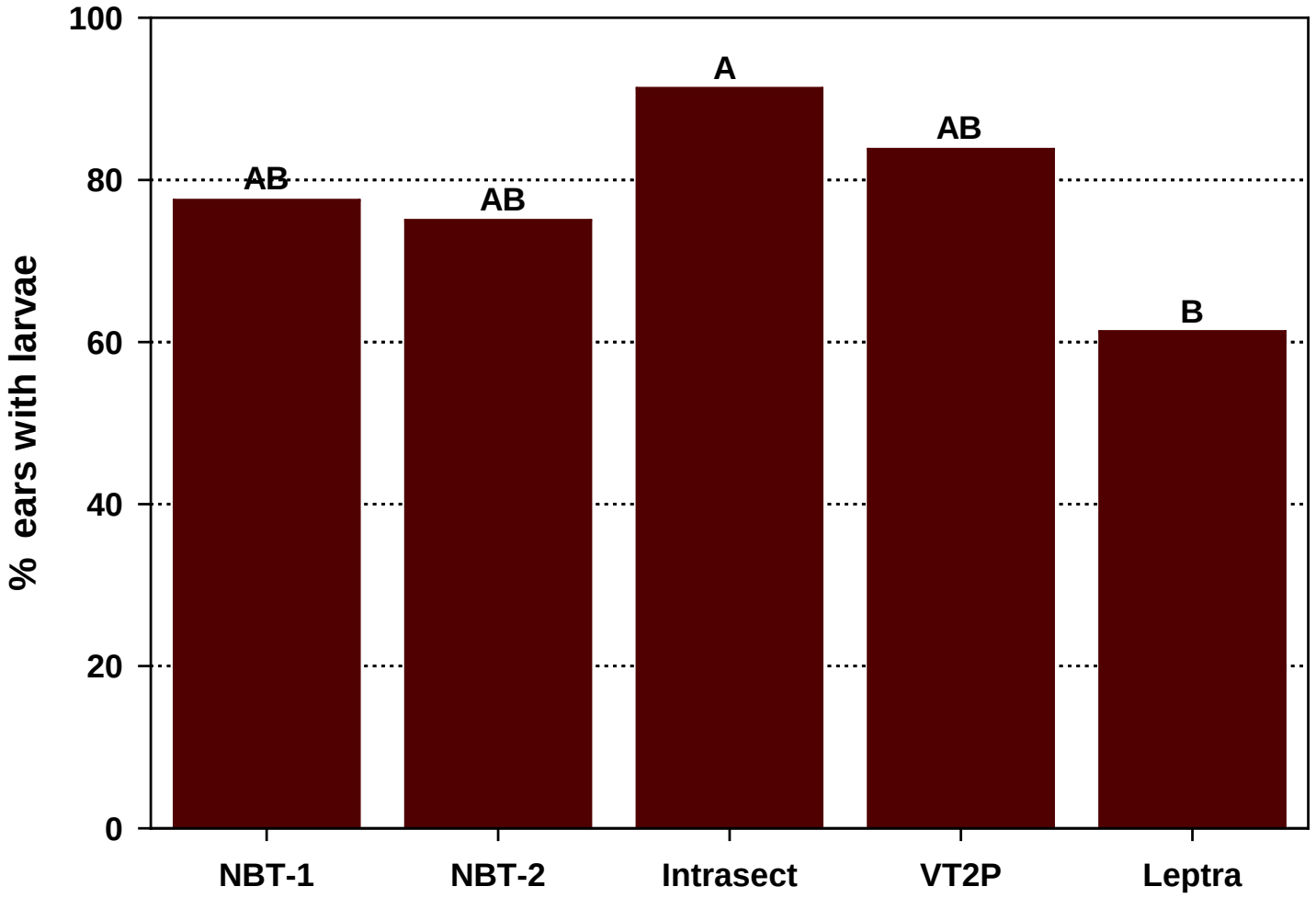
- Most common are incidences where there is unacceptable injury (20-30%) but very little worm survival
- The reason for survival to large worms is not known
- May result from low Bt expression, resistance or both

INCIDENCES OF BOLLWORM INJURY IN TRIPLE GENE COTTON

RESISTANCE RATIOS: CRY2Ab2 = 3.46, VIP3Aa39 = 3.00

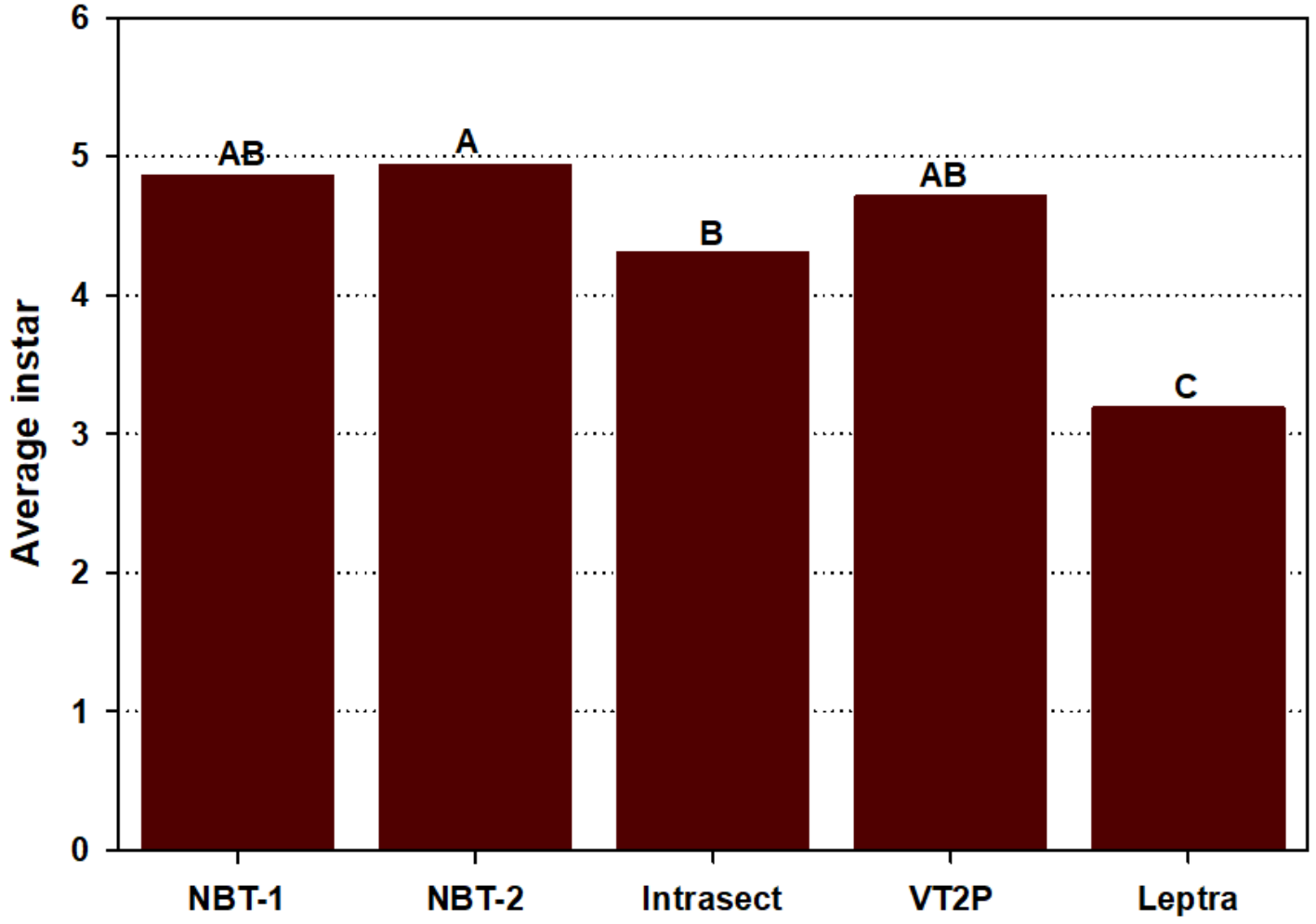
BT CORN TRAIT PERFORMANCE

FIELD PERFORMANCE OF *H. ZEA* ON DIFFERENT CORN TECHNOLOGIES



Technology	Bt traits
NBT-1&2	None
Intrasect	Cry1Ab+Cry1F
VT2P	Cry1A.105+Cry2Ab2
Leptra	Cry1Ab+Cry1F+Vip3A

FIELD PERFORMANCE OF *H. ZEA* ON DIFFERENT CORN TECHNOLOGIES



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NBT-1&2	None
Intrasect	Cry1Ab+Cry1F
VT2P	Cry1A.105+Cry2Ab2
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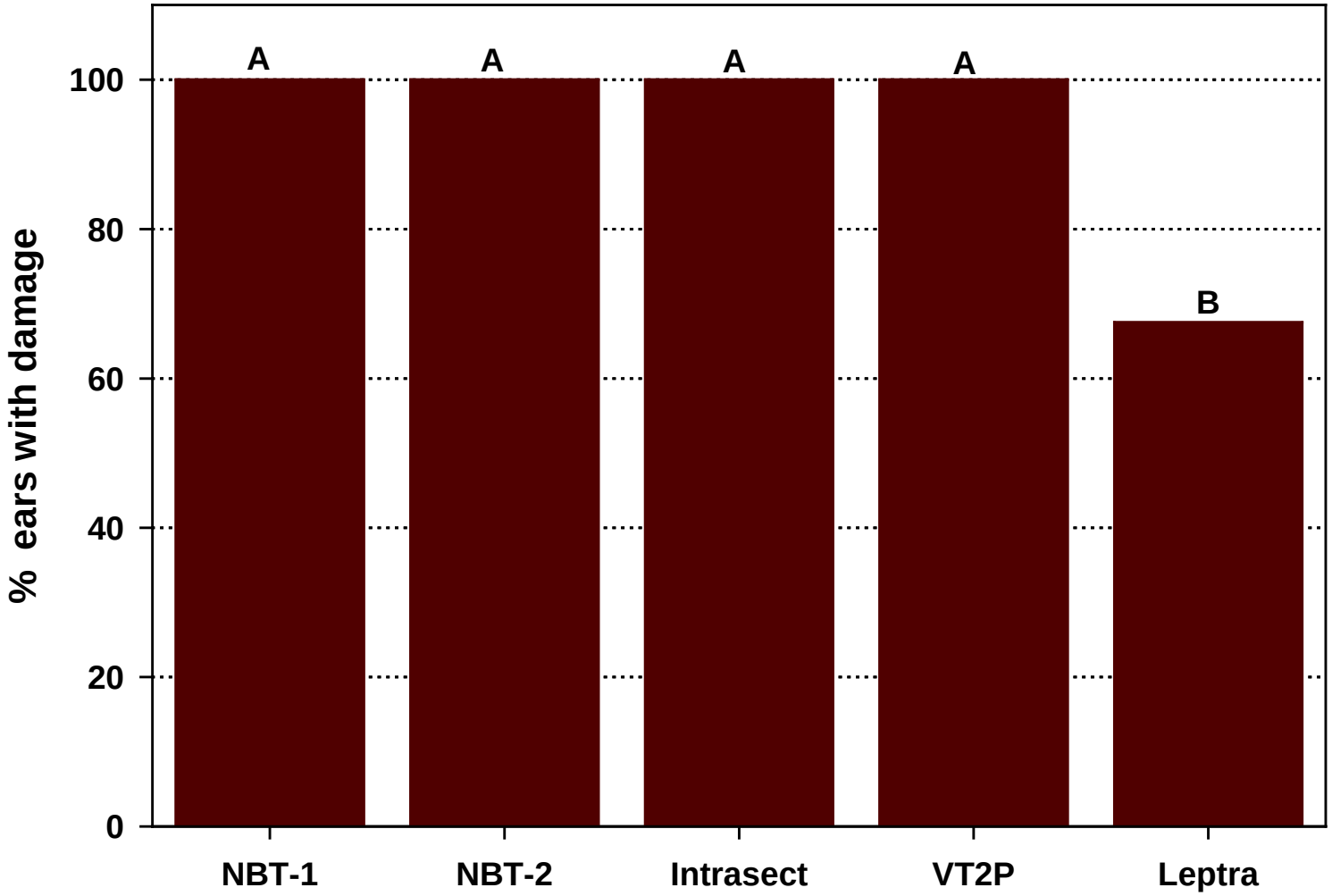
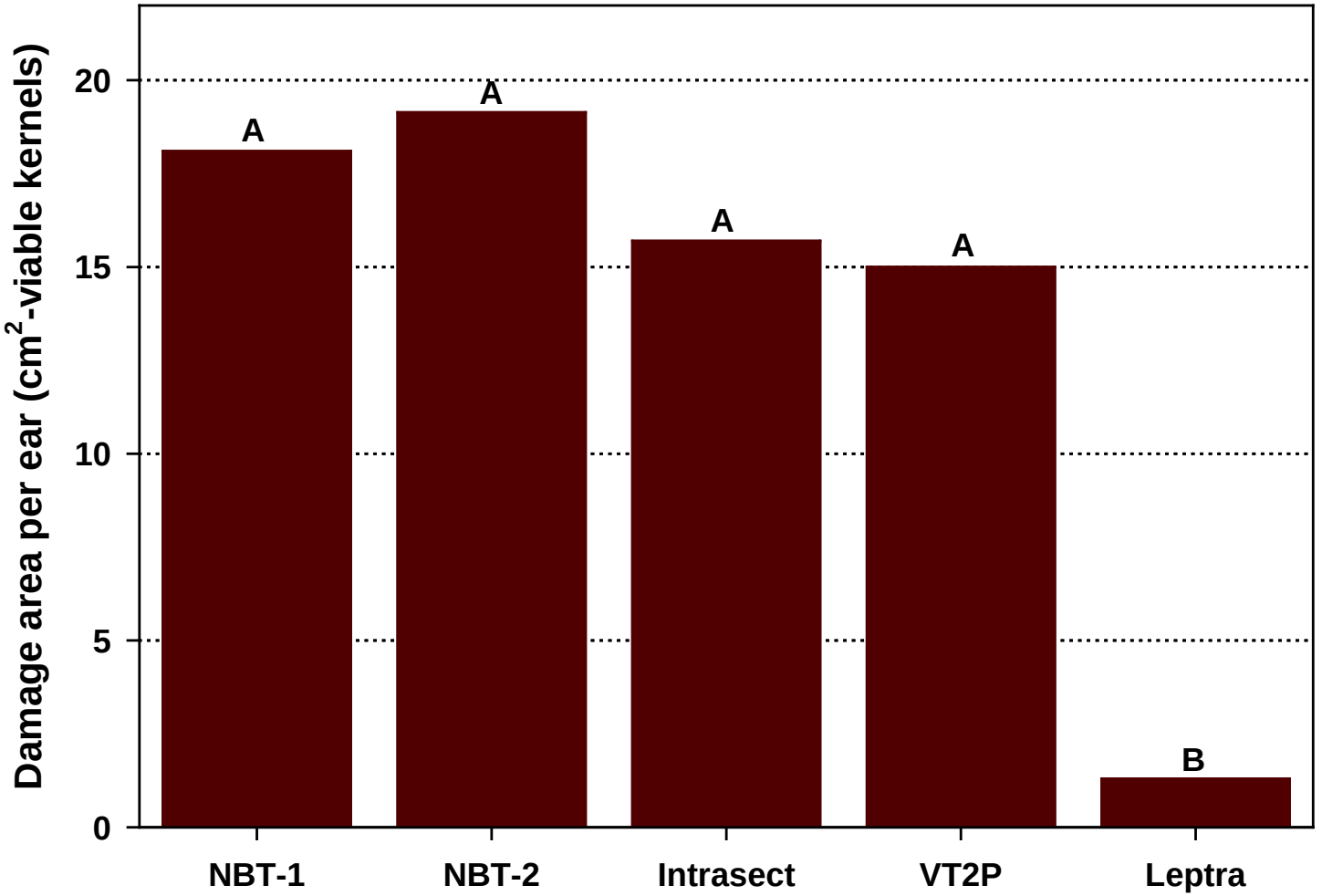


Technology	Bt traits
NBT-1&2	None
Intrasect	Cry1Ab+Cry1F
VT2P	Cry1A.105+Cry2Ab2
Leptra	Cry1Ab+Cry1F+Vip3A

FIELD PERFORMANCE OF *H. ZEA* ON DIFFERENT CORN TECHNOLOGIES



Technology	Bt traits
NBT-1&2	None
Intrasect	Cry1Ab+Cry1F
VT2P	Cry1A.105+Cry2Ab2
Leptra	Cry1Ab+Cry1F+Vip3A

CORN EARWORM FEEDING IN LEPTRA CORN IN MS -2019

J. Gore & D. Cook MSU

PROTEIN BIOASSAY-OVERLAY

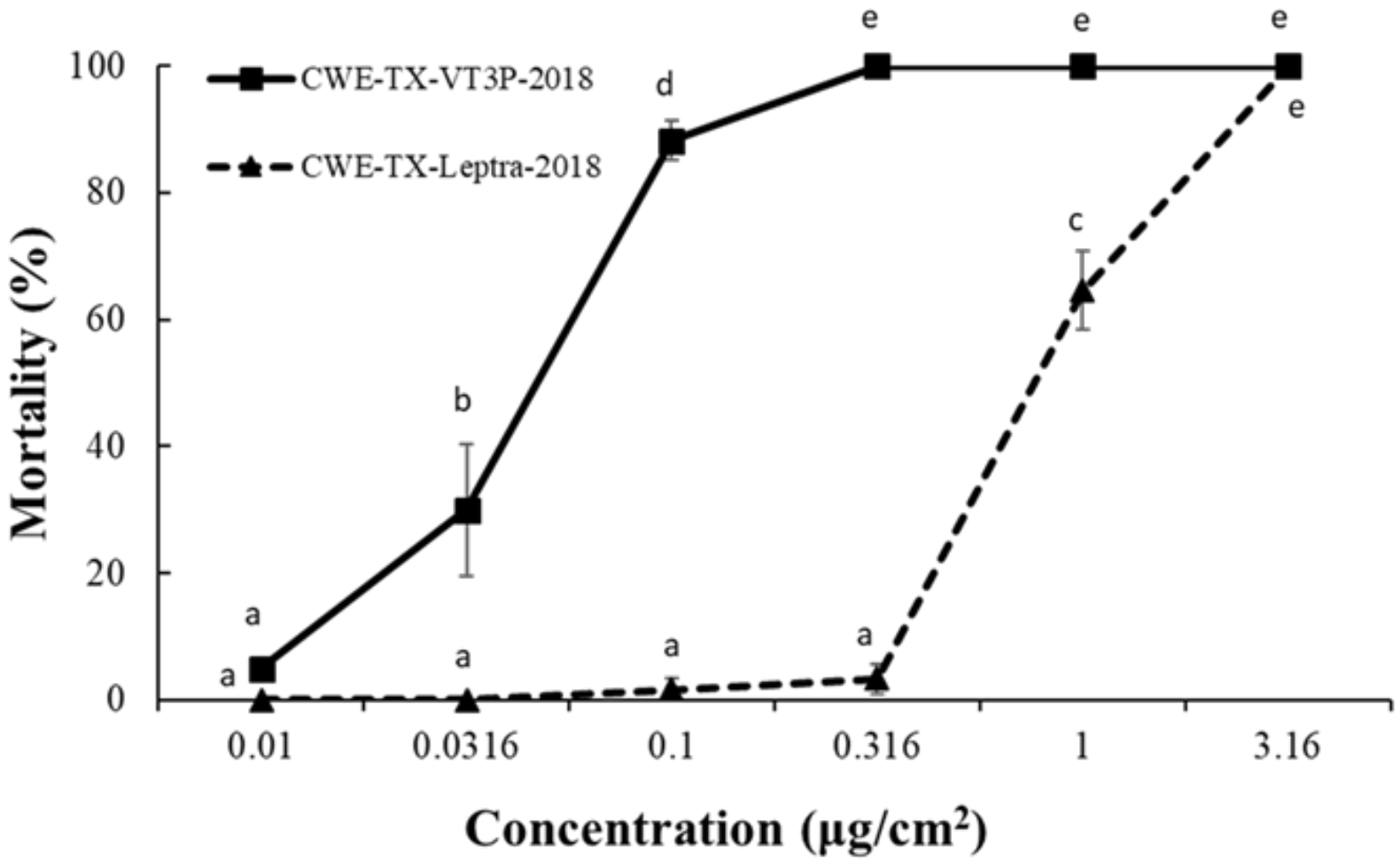
Insects: 1) CEW-TX-Leptra-2018, collected from Leptra (Cry1Ab+Cry1F+Vip3A)

2) CEW-TX-VT3P-2018, collected from VT3P (Cry1F+Cry1A.105+Cry2Ab2)

Diet bioassays:

- 128-CD- International trays
- Vip3A1 protein provided by BASF
- 7-8 concentrations and a control
- 1 neonate/cell; 4 replications;16 insects/rep.
- 28 °C, ~50% RH and photoperiod of 16:8(L:D)h.
- Larval instar & mortality were recorded after 7d.

MORTALITY


CWE-TX-VT3P-2018
CWE-TX-Leptra-2018
Mortality (%)
100
80
60
40
20
0
0.01
0.0316
0.1
0.316
1
3.16
Concentration (µg/cm²)
a
a
b
a
d
a
e
a
e
c
e
e

LC_{50}S / RESISTANCE RATIOS

Insect population*	N#	LC_{50} (95% CI) (µg/cm²) §	Slope ± SE	X^2	df	Resistance ratio£
CEW-TX-VT3P-2018	448	0.041 (0.035, 0.050)	2.87 ± 0.30	18.9	22	1.0
CEW-TX-Leptra-2018	448	0.838 (0.686, 0.966)	4.93 ± 1.02	19.0	22	20.4

COTTON LEAF BIOASSAY

Cotton varieties: Widestrike 3 (WS3), expressing Cry1F+Cry1Ac+Vip3A protein and non-Bt.

Insects: 1) CEW-TX-Leptra-2018, collected from Leptra (Cry1Ab+Cry1F+Vip3A)

2) CEW-TX-SS, lab susceptible strain

Leaf tissue bioassays:

- Cotton planted in the field
- Cotton leaves excised at the 7-8 nodes growth stage
- Expression/non-expression of the Cry and Vip3A proteins using stripes
- Leaves were placed in Petri Dishes lined with moistened filter paper
- 5 neonate/dish; 4 replications; 30 insects/rep.
- 28 °C, ~50% RH and photoperiod of 16:8(L:D)h.
- Larval instar & survivorship were recorded after 7d.

COTTON LEAF ASSAY RESULTS

Cotton variety	Insect	Survivorship (%)	Average instar
Non-Bt	CEW-TX-Leptra-2018	78.3 ± 2.9 c	3.39 ± 0.01 c
	CBW-TX-SS	82.5 ± 3.2 c	3.45 ± 0.05 c
WideStrike 3	CEW-TX-Leptra-2018	41.7 ± 7.5 b	2.68 ± 0.03 b
	CBW-TX-SS	3.3 ± 1.4 a	2.00 ± 0.00 a

COLLECTIONS FROM VIP-TRAITED CROPS

	Cry1Ac		Cry2Ab2		Vip3Aa39	
Insect strain	Resistance ratio-BZ	Resistance ratio-TX	Resistance ratio-BZ	Resistance ratio-TX	Resistance ratio-BZ	Resistance ratio-TX
CBW-BZ-SS-TAMU	1.0	-1.2	1.0	-1.1	1.0	4.05
CBW-TX-SS-TAMU	1.2	1.0	1.1	1.0	-4.05	1.0
CBW-MS-SE-Leptra	21.55	26.33	3.25	2.93	3.20	13.00
CBW-TX-SK-Leptra	5.18	6.33	3.64	3.27	-1.05	3.88
CBW-TX-SK-BG3	99.27	121.33	3.46	3.11	-1.35	3.00

F2 SCREENS FOR RESISTANT ALLELES

LARVAL COLLECTION METHOD F_2 SCREEN

Andow, D. A., and D. N. Alstad. 1998.

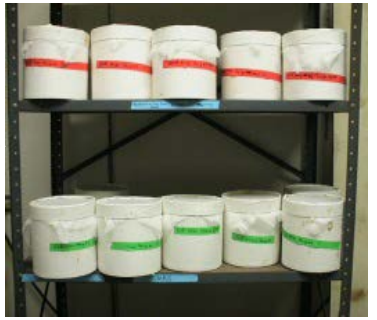
Larval collection and rearing

Feral ♂ x susceptible (SS) ♀

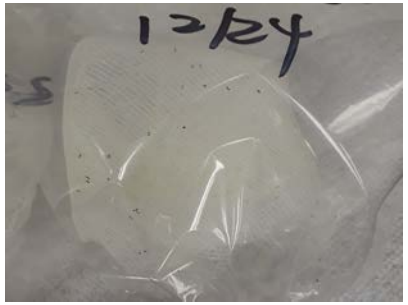
Establishing Isoline families

F_1 family

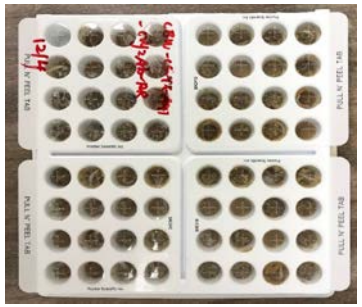
F_1 families are reared and Sib mated



F_2 family



F_2 screen with discriminatory [ ] of *Bt* toxins



Check results after 7 days

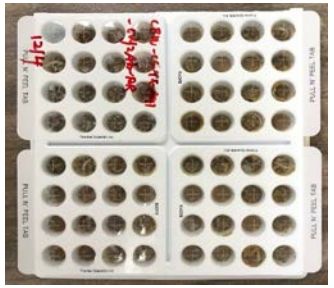
F_2


(SS) 56.25%


(RS) 37.5%

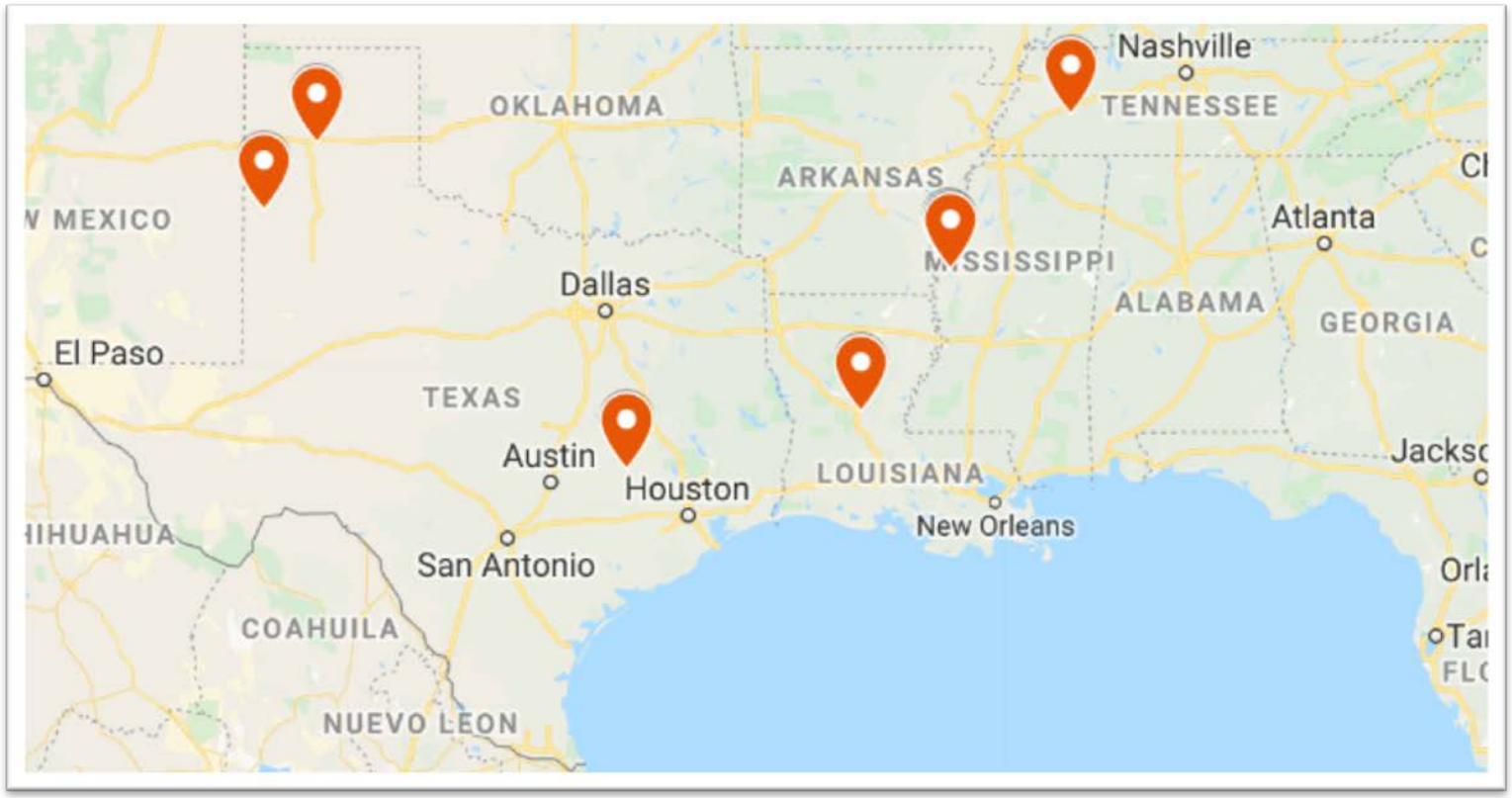

(RR) 6.25%

Assuming resistance controlled by a single gene and recessive alleles



Full range bioassay for resistance confirmation

INSECT SOURCES



- Feral *H. zea* (moths and larvae) were collected from major *Bt* transgenic cotton and corn production regions of the southern US (April to October 2018 and 2019)

BIOASSAYS

Discriminatory concentrations (Yang et al., 2017)

- Cry1Ac (**10 µg/cm^2**) (Bayer Crop Science)
- Cry2Ab2 (**10 µg/cm^2**) (Bayer Crop Science)
- Vip3Aa51 (**3 µg/cm^2**) (BASF)
- One F_2 neonate (<24 h) was placed per well 128 observations/F_2 family/toxin
- Survival was recorded after 7 days of exposure to the toxin
- Potentially resistant families were defined as those resulting in 5 survivors with at least 1 larva ≥ 3rd instar

H. zea-F_2 FAMILIES SURVIVING THE DIAGNOSTIC CONCENTRATION OF CRY1Ac (10 µg/cm^2)

				7-day survival	
Location	**Collection date**	**Method to establish the F_2 families**	**Number of F_2 families screened**	**Number of surviving families***	**Number of 3rd and 4th instar larvae**
Texas	April-August, 2018 and 2019	Light trap/ Cross with SS♀	106	98	3695
Louisiana	June, 2019	Cross with SS♀	21	19	313
Mississippi	July, 2019	Cross with SS♀	15	15	535
Tennessee	July, 2019	Cross with SS♀	6	6	217
**5 survivors with at least 1 larva ≥ 3rd instar*		**Total**	148	138 **(93%)**	4760

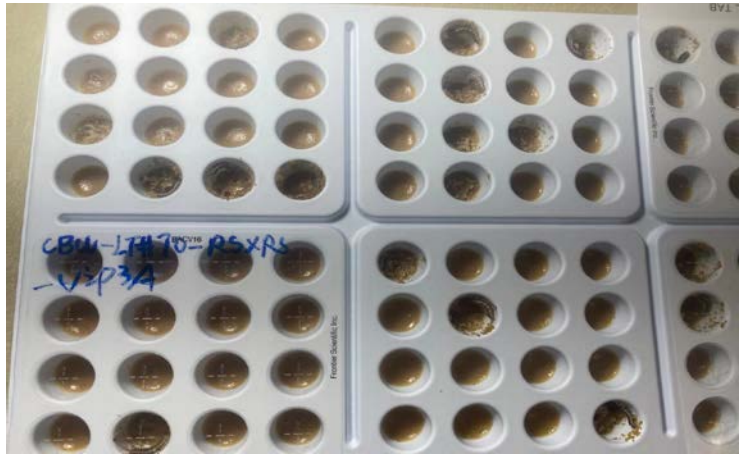
H. zea-F_2 FAMILIES SURVIVING THE DIAGNOSTIC CONCENTRATION OF CRY2Ab2 (10 µg/cm^2)

				7-day survival	
Location	**Collection date**	**Method to establish the F_2 families**	**Number of F_2 families screened**	**Number of surviving families***	**Number of 3rd and 4th instar larvae**
Texas	April-August, 2018 and 2019	Light trap/ Cross with SS♀	120	47	273
Louisiana	June, 2019	Cross with SS♀	21	4	22
Mississippi	July, 2019	Cross with SS♀	15	11	75
Tennessee	July, 2019	Cross with SS♀	6	1	2
**5 survivors with at least 1 larva ≥ 3rd instar*		**Total**	162	63 **(39%)**	372

H. zea-F_2 FAMILIES SURVIVING THE DIAGNOSTIC CONCENTRATION OF VIP3Aa51 (3 µg/cm^2)

				7-day survival	
Location	**Collection date**	**Method to establish the F_2 families**	**Number of F_2 families screened**	**Number of surviving families***	**Number of 3rd and 4th instar larvae**
Texas	April-August, 2018 and 2019	Light trap/ Cross with SS♀	126	1	7
Louisiana	June, 2019	Cross with SS♀	21	1	21
Mississippi	July, 2019	Cross with SS♀	15	0	0
Tennessee	July, 2019	Cross with SS♀	6	0	0
**5 survivors with at least 1 larva ≥ 3rd instar*		**Total**	168	2 **(1.2%)**	28

IDENTIFICATION OF VIP3A RESISTANT GENES IN H. ZEA



CBW-TX-LT#70-Vip3Aa-RR

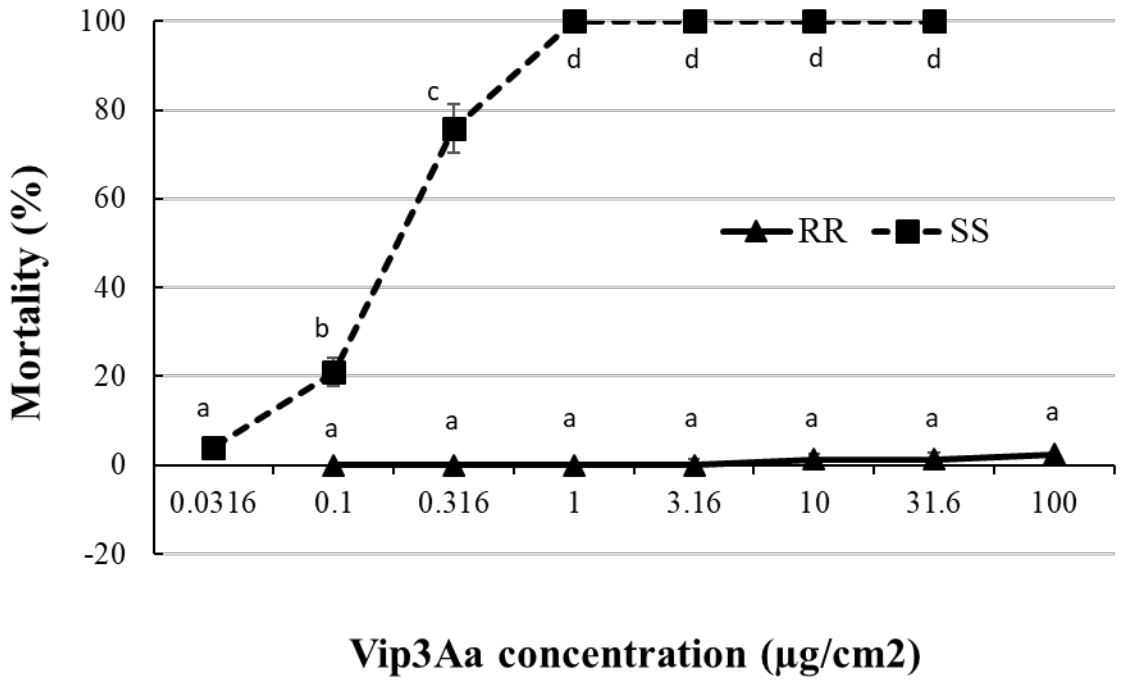
RS*RS selected on 30 ug/cm^2 of Vip3Aa

Insect strain	Vip3Aa concentration	No. insects assayed	Observed survivor		Expected survivor
RS*RS	30 ug/cm^2	512	2nd	8	512*25% of RR = 128
			3rd	8	
			≥4th	111	
			Total	127	

Based on percentage survivorship, the Vip resistance appears to be controlled by a single recessive gene

Table 1. Mortality response (LC_{50}) of different genotypes of *Helicoverpa zea* to Vip3Aa39 toxins in diet-overlay bioassays.

Bt protein	Population	N	LC_{50} (95% CL) (µg/cm^2)	Slope ± SE	X^2	df	Resistance ratio
Vip3Aa39	CBW-TX-SS	512	0.17 (0.14, 0.21)	2.84 ± 0.29	10.5	26	-
	CBW-TX-LT#70-Vip3Aa-RR	512	2.348E20	/	/	/	1.38E21



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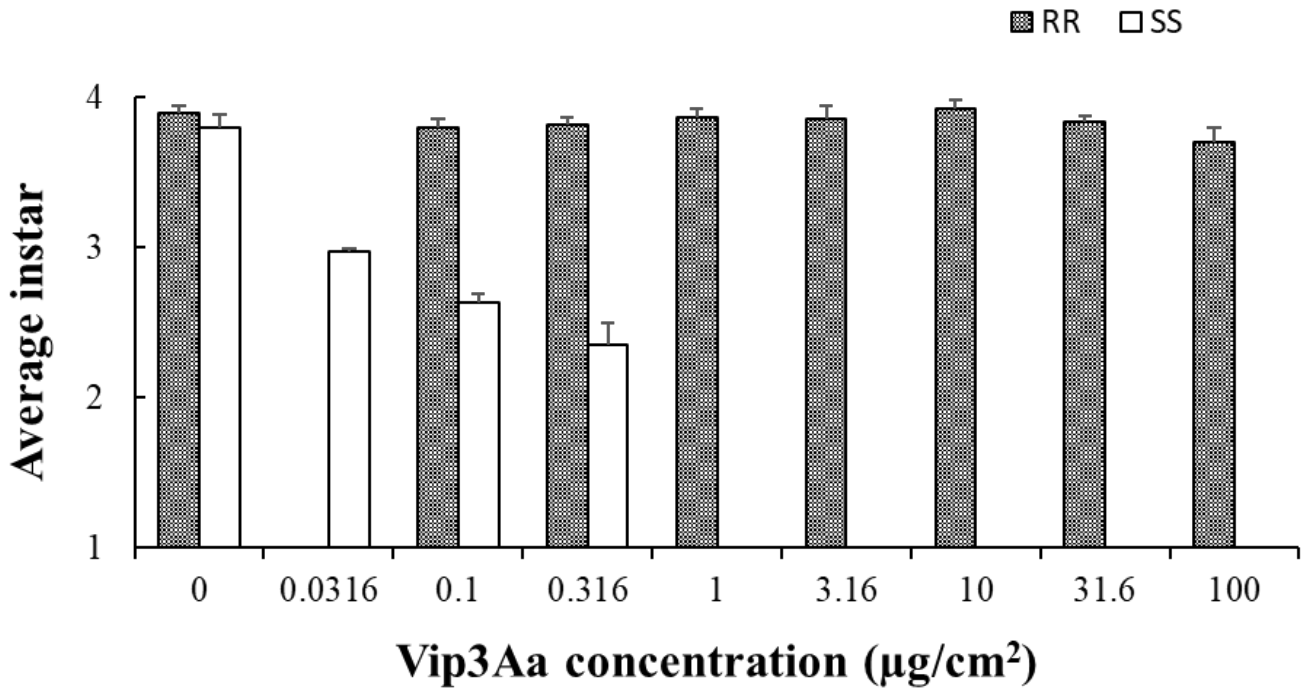
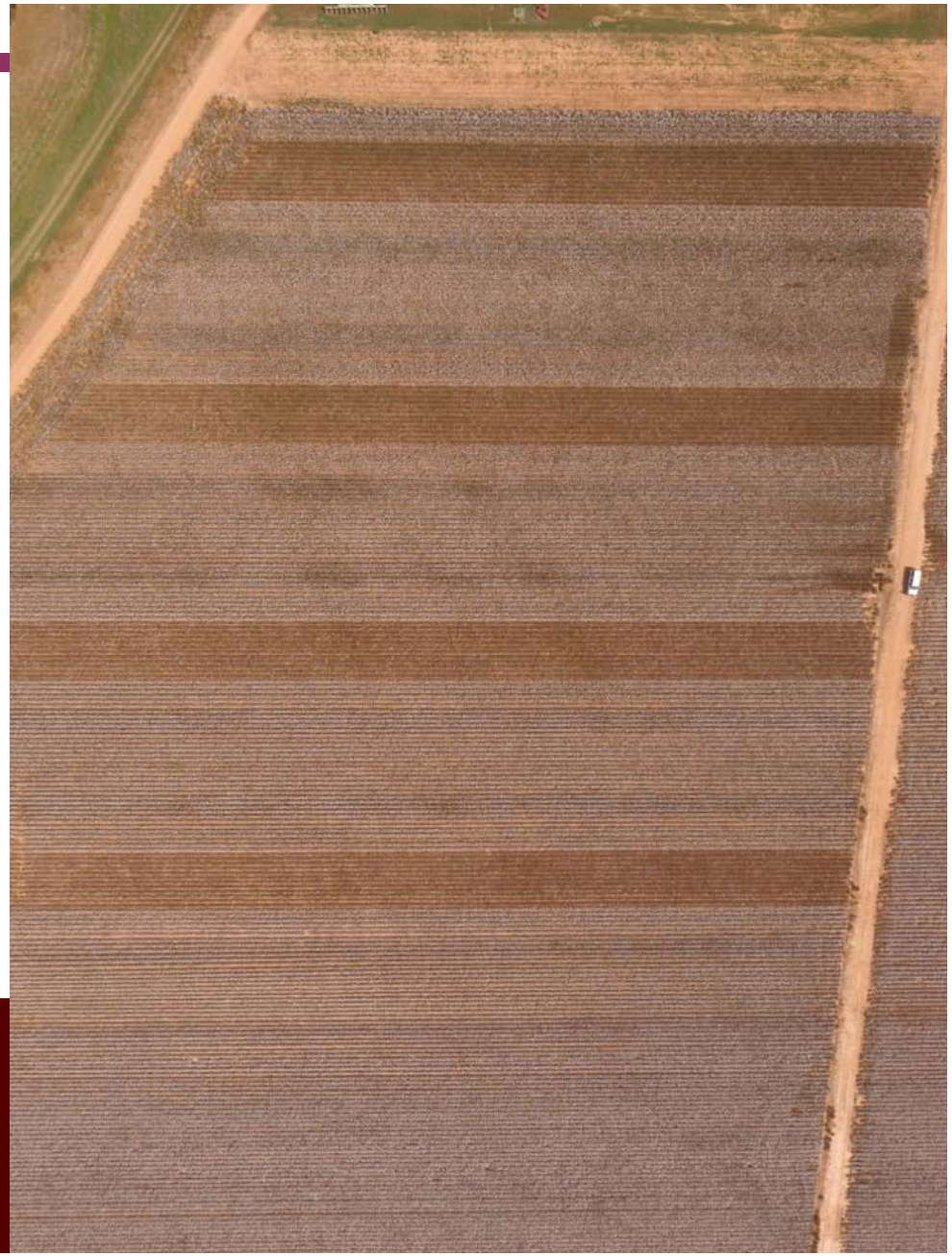
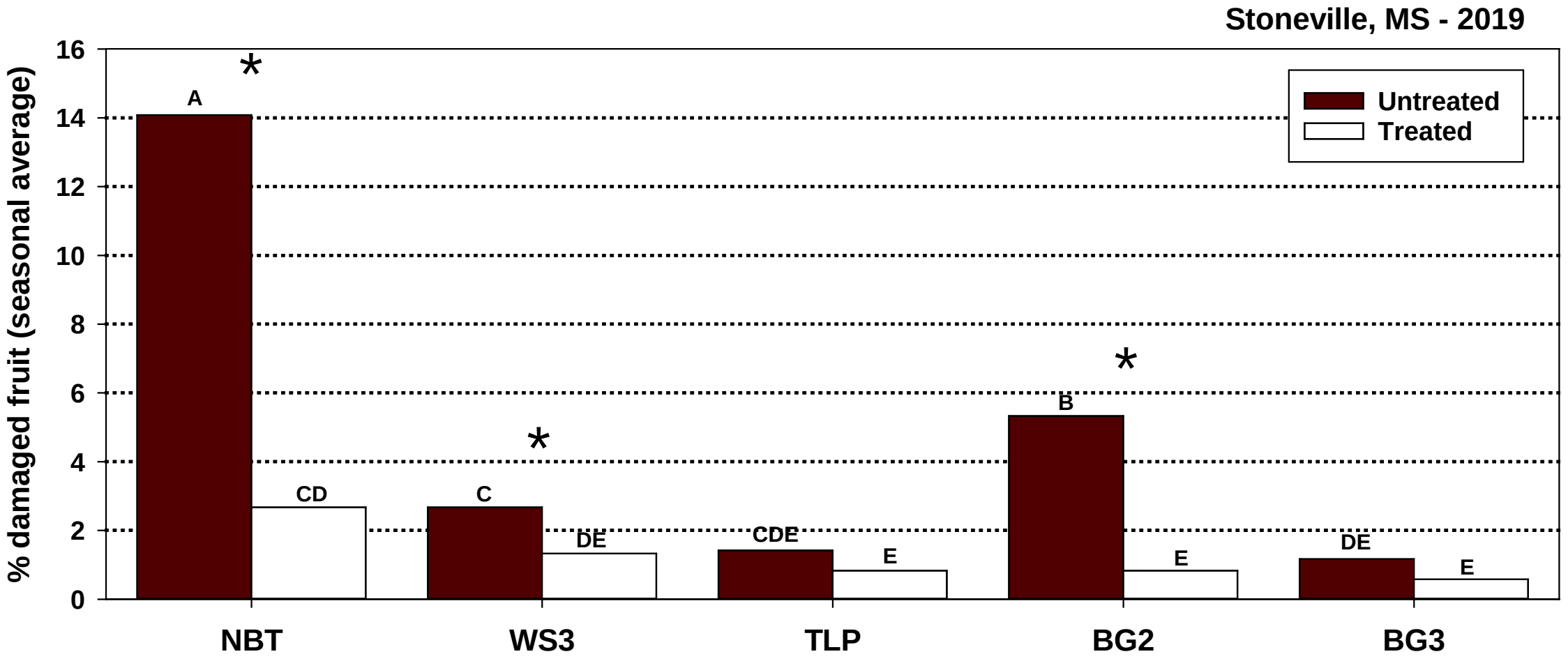
Vip3Aa39	CBW-TX-SS	512	0.17 (0.14, 0.21)	2.84 ± 0.29	10.5	26	-
	CBW-TX-LT#70-Vip3Aa-RR	512	2.348E20	/	/	/	1.38E21



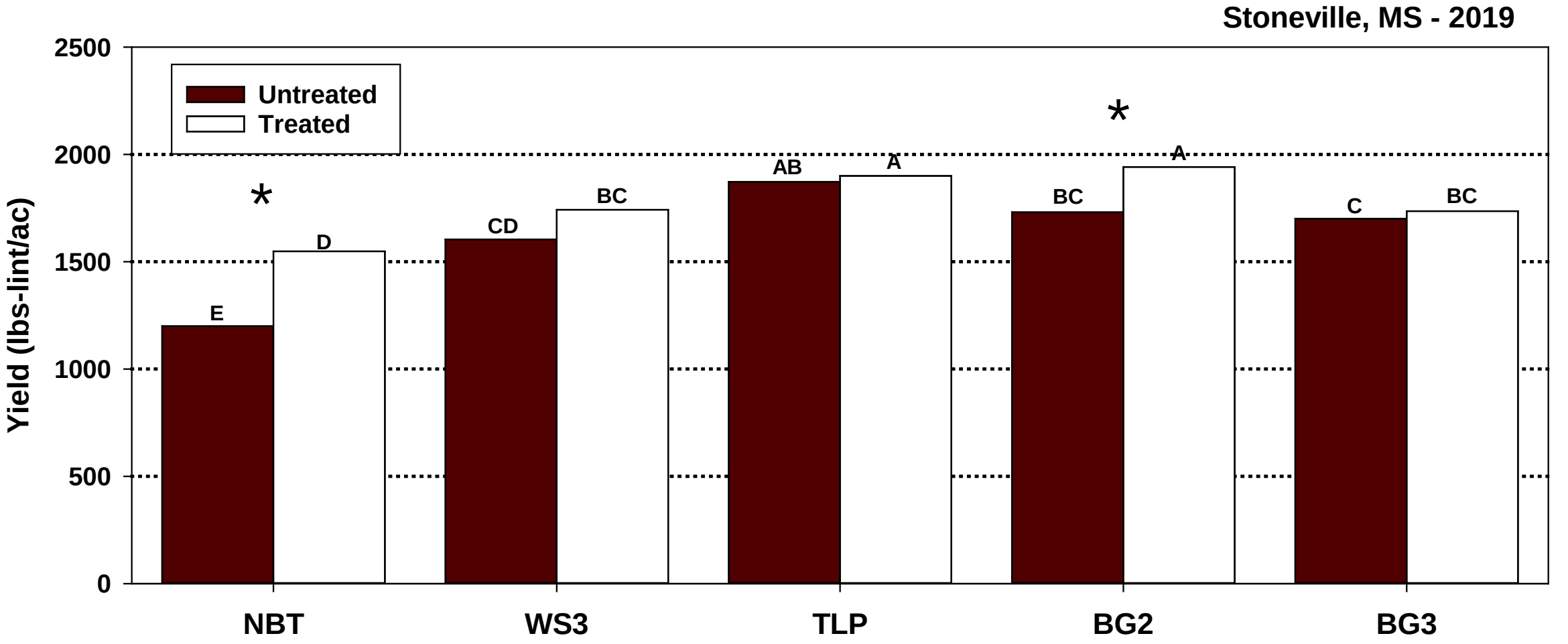
FIELD PERFORMANCE

EFFICACY OF BT COTTON TECHNOLOGIES AND VALUE OF TREATING WITH INSECTICIDE





J. Gore, Mississippi State



J. Gore, Mississippi State

QUESTIONS?

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