BOLLWORM RESISTANCE IN BT 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)
Monsanto	Bollgard (Cry1Ac)	Bollgard 2 (Cry1Ac+Cry2Ab)		Bollgard 3 (Cry1Ac+Cry2Ab+Vip3A)
Dow		WideStrike (Cry1Ac+Cry1F)	WideStrike 3 (Cry1Ac+Cry1F+Vip3A)	
Bayer		TwinLink (Cry1Ab+Cry2Ae)		TwinLink Plus (Cry1Ab+Cry2Ae+Vip3A) A)

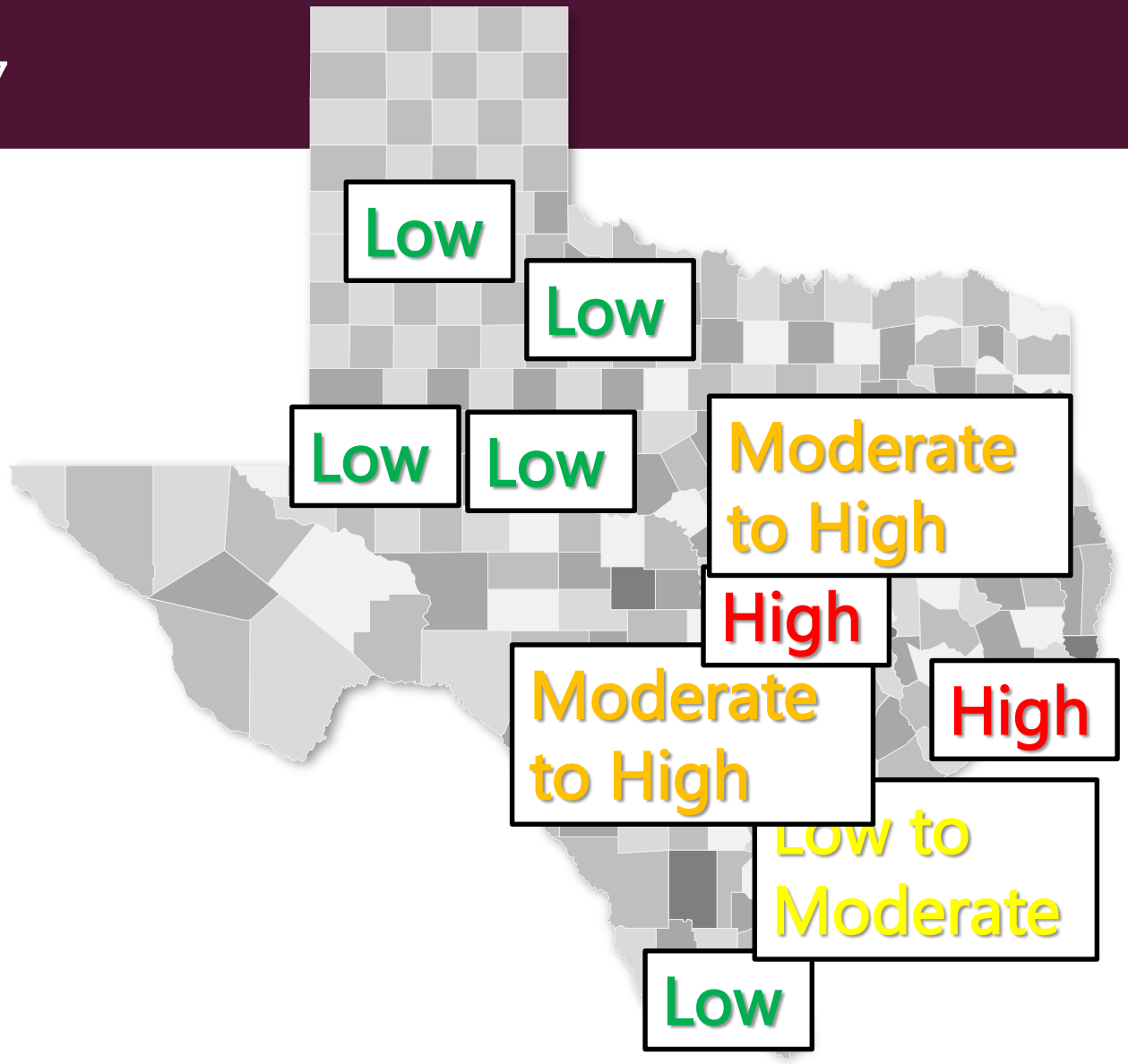
Homogeny across crops				
Crop	**Cry1A**	**Cry1F**	**Cry2**	**Vip3A**
Cotton	Cry1Ac, Cry1Ab	Cry1F	Cry2Ab, Cry2Ae	Vip3A
Corn	Cry1Ab	Cry1F	Cry2Ab2	Vip3A
	Cry1A.105 (Cry1Ab, Cry1Ac, Cry1F)			

THE CURRENT SITUATION

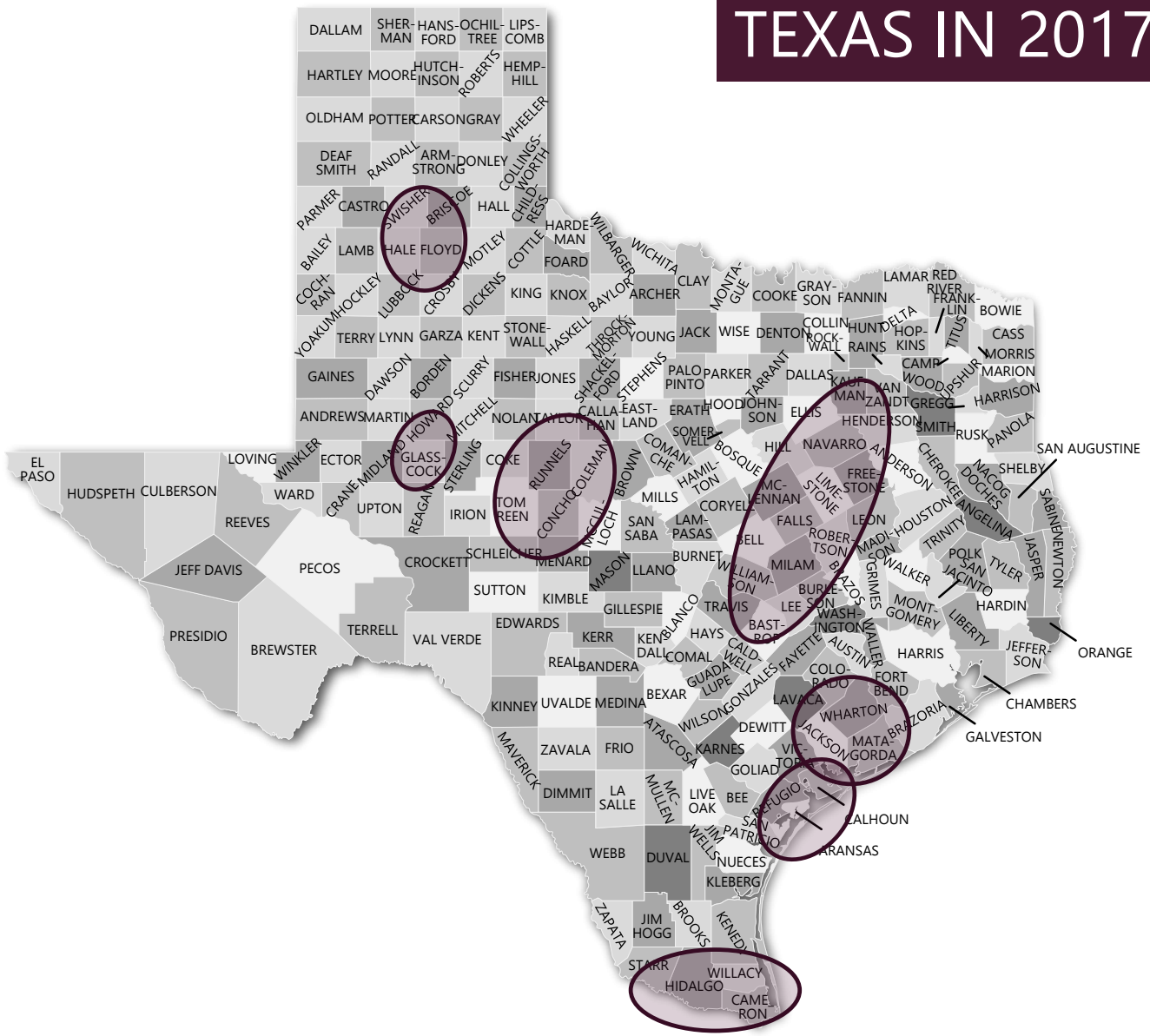
ARE BT TECHNOLOGIES PROVIDING THE CONTROL WE HAVE COME TO EXPECT

BOLLWORM PRESSURE IN 2017

Variable bollworm pressure across the state



HOW MUCH BT COTTON WAS TREATED IN TEXAS IN 2017?

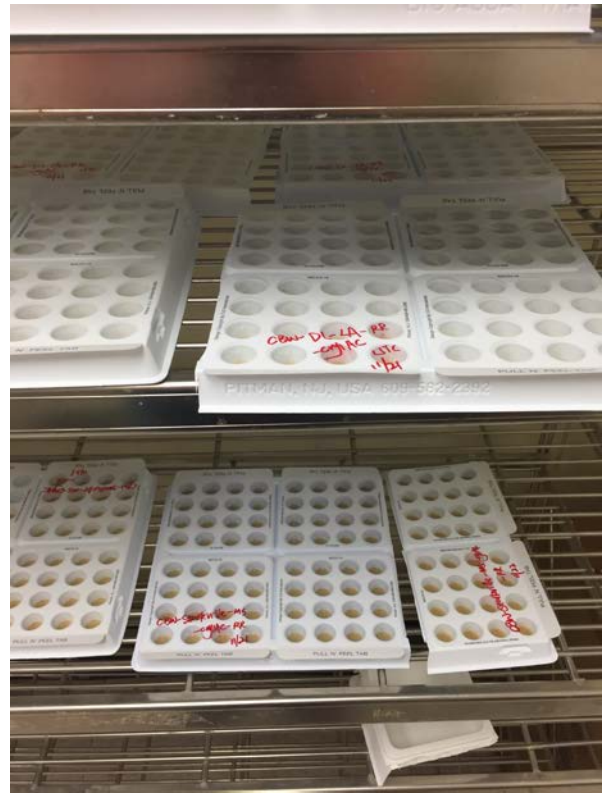


Primarily the problem with Bt cotton efficacy appears to be most prevalent along the Gulf Coast and the Blacklands in areas with higher bollworm pressure

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 eight Bt concentrations and a control
  - Use 32 neonate larvae, replicated 4 times for each concentration (1024 larvae per Bt toxin)
- Allowed to feed for 7 days
- Record number alive/dead, instar and weight of each survivor
- Compare field populations to a standard laboratory strain (Benzon)
  - Dead = Actual dead larvae + 1st and 2nd instar larvae

DIET OVERLAY BIOASSAYS (CRY1AC, CRY2AB2 & VIP3A)



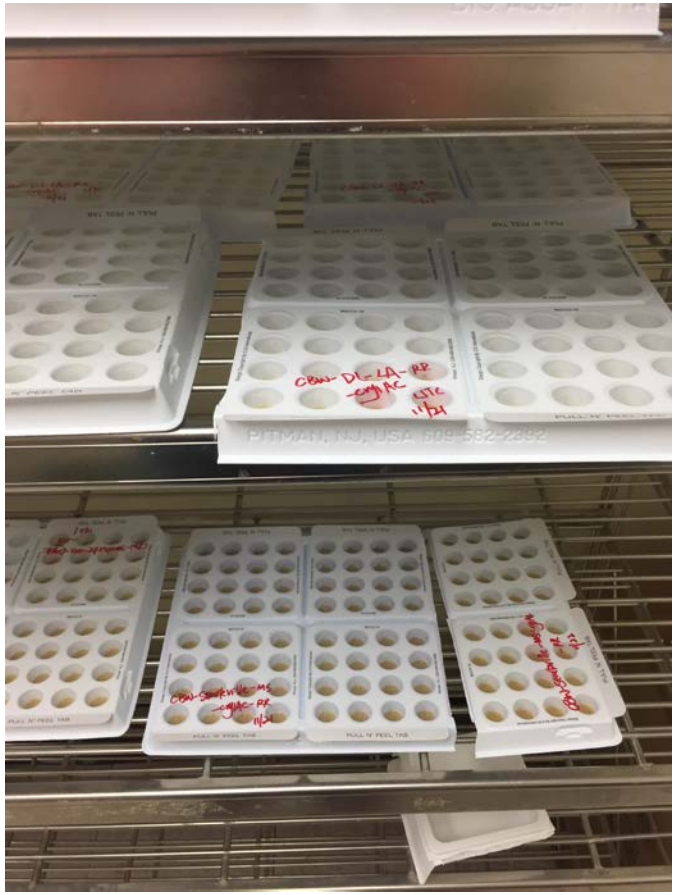
Diet-overlay concentrations of 0, 0.01, 0.0316, 0.1, 0.316, 1.0, 3.16, 10.0 and 31.6 µg/cm².

WHAT CONSTITUTES RESISTANCE?

- There is naturally occurring variation in response to Bt toxins
- Field-evolved (or field-selected) resistance as a genetically based decrease in susceptibility of a population to a toxin caused by exposure of the population to the toxin in the field
- Field-evolved resistance can be demonstrated directly by showing decreases in susceptibility to a toxin over time
- More commonly, field-evolved resistance is documented indirectly by showing that one or more field populations with a history of exposure to toxin are less susceptible than conspecific field populations or laboratory strains that have had little or no such exposure
  - Resistance ratios >10 are more likely to reflect genetically based decreases in susceptibility
- Four categories of field-evolved resistance:
  - 1) >50% resistant individuals and reduced efficacy of the Bt crop in the field has been reported
  - 2) >50% resistant individuals and reduced efficacy is expected, but has not been reported
  - 3) 1–6% resistant individuals (reduced efficacy of the Bt crop in the field is not expected)
  - 4) <1% resistant individuals (reduced efficacy of the Bt crop in the field is not expected)

BIOASSAY DATA

CRY1AC, CRY2AB2 AND VIP3A



CRY1AC DATA - 2015

Insect strain	Generation	LC50 (95% CL) (µg/g)	Resistance ratio
USDA-SS	/	0.265 (0.207, 0.339)	1
WB-LA	G1	1.340 (1.038, 1.738)	5.1
BR-LA	G2	> 10	> 37.7
AD-LA	G2	> 10	> 37.7
SV-MS	G1	> 10	> 37.7
SD-MS	G2	6.760 (3.856, 15.443)	25.5
MT-AR	G2	1.291 (1.024, 1.655)	4.9

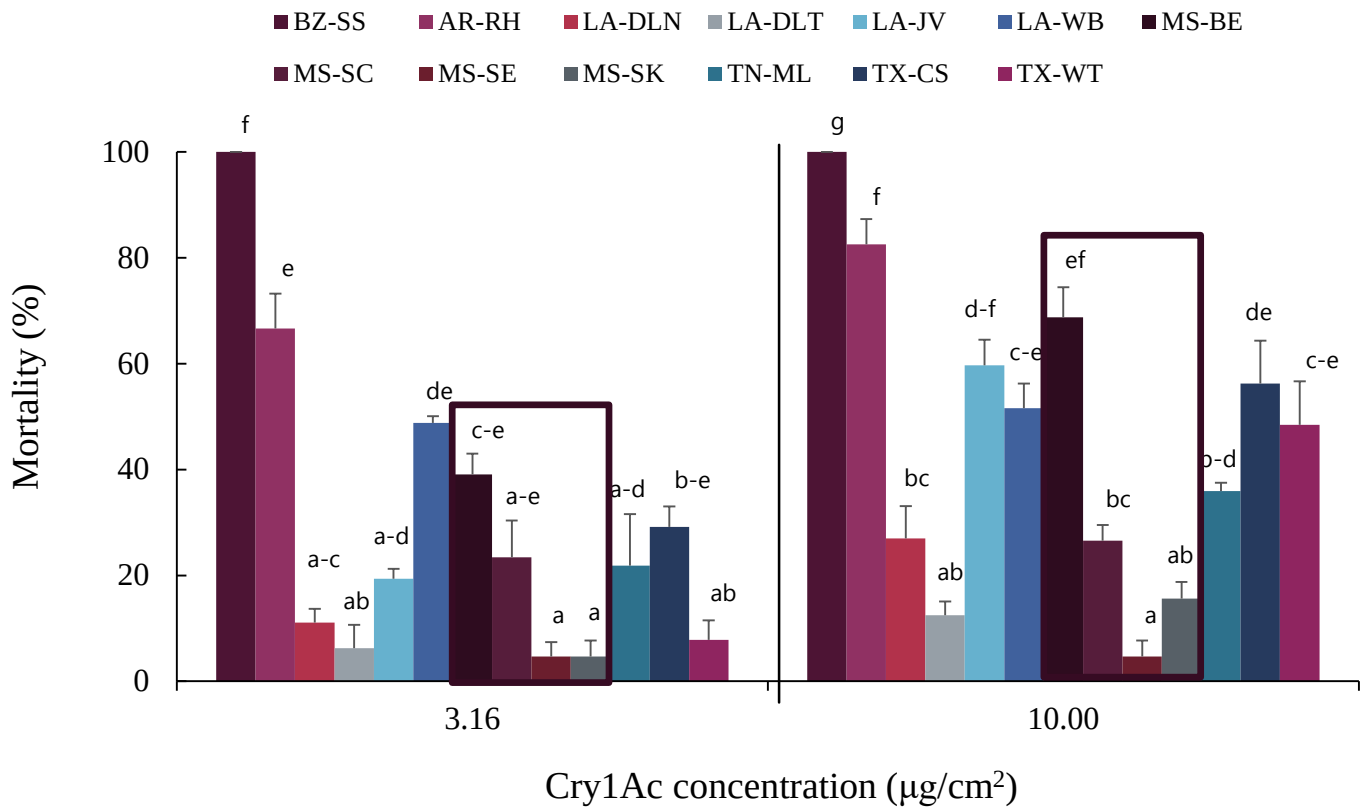
CRY1AC DATA - 2016

Insect strain	LC_{50}-1 (95% CL) ($\mu g/cm^2$)	RR-1	LC_{50}-2 (95% CL) ($\mu g/cm^2$)	RR-2
BZ-SS	0.027 (0.023, 0.031)	1.0	0.015 (0.012, 0.017)	1.0
LA-AD	0.942 (0.575, 1.611)	34.9 *	0.412 (0.270, 0.620)	27.5 *
TN-JN	0.202 (0.096, 0.394)	7.5	0.086 (0.038, 0.163)	5.7
TN-BG2	0.237 (0.193, 0.292)	8.8	0.143 (0.109, 0.185)	9.5
MS-LD	1.341 (0.967, 1.930)	49.7 *	0.725 (0.534, 1.004)	48.3 *
AR-TK	0.057 (0.041, 0.075)	2.1	0.024 (0.013, 0.038)	1.6

LC_{50} and 95% confidence limits (CL) based on larval mortality of *Helicoverpa zea* to Cry1Ac in 2017.

Insect strain	Host	Traits	N[a]	LC_{50} (95% CL) (µg/cm^2)[b]	Slope ± SE	X^2	df	Resistance ratio[c]
BZ-SS	Laboratory	/	958	0.091 (0.077, 0.108)	1.55 ± 0.09	28.4	26	1.0
LA-WB-NBt corn	NBT-Corn	/	948	6.259 (2.000, 70107)	1.38 ± 0.59	46.2	26	68.8 *
TX-CS-WS	Widestrike-Cotton	Cry1Ac, Cry1F	958	> 10.000	/	/	/	> 109.8 *
TX-Wharton-TL	Twinlink-Cotton	Cry1Ab, Cry2Ae	945	> 10.000	/	/	/	> 109.8 *
MS-Stoneville-VT2P	Double Pro-Corn	Cry1A.105, Cry2Ab2	952	> 10.000	/	/	/	> 109.8 *
LA-DL-NBt cotton	NBt-Cotton	/	512	> 10.000	/	/	/	> 109.8 *
MS-Starkville-VT2P	Double Pro-Corn	Cry1A.105, Cry2Ab2	512	> 10.000	/	/	/	> 109.8 *
AR-Rohwer-GS	Grain sorghum	/	512	2.771 (0.368, 13033)	1.57 ± 0.72	53.0	26	30.5 *
MS-Silver City-BG2	Bollgard 2-Cotton	Cry1Ac, Cry2Ab	512	> 10.000	/	/	/	> 109.8 *
LA-DL-TL	Twinlink-Cotton	Cry1Ab, Cry2Ae	512	> 10.000	/	/	/	> 109.8 *
LA-Jonesville-BG2	Bollgard 2-Cotton	Cry1Ac, Cry2Ab	512	> 10.000	/	/	/	> 109.8 *
MS-Benoit-BG2	Bollgard 2-Cotton	Cry1Ac, Cry2Ab	512	5.645 (3.621, 10.141)	0.94 ± 0.10	32.0	26	62.0 *
TN-Milan-VT2P	Double Pro-Corn	Cry1A.105, Cry2Ab2	512	> 10.000	/	/	/	> 109.8 *
TN-Jackson-Obsession II	Obsession II-Sweet corn	Cry1A.105, Cry2Ab2	512	> 10.000	/	/	/	> 109.8 *
AR-Pine Bluff-Bt cotton	Bt-Cotton, multiple	Varied	512	> 10.000	/	/	/	> 109.8 *

LC_{50} of Dead larvae + 1st instar larvae + 2nd instar larvae. Resistance ratio = LC_{50} of field population / LC_{50} of laboratory

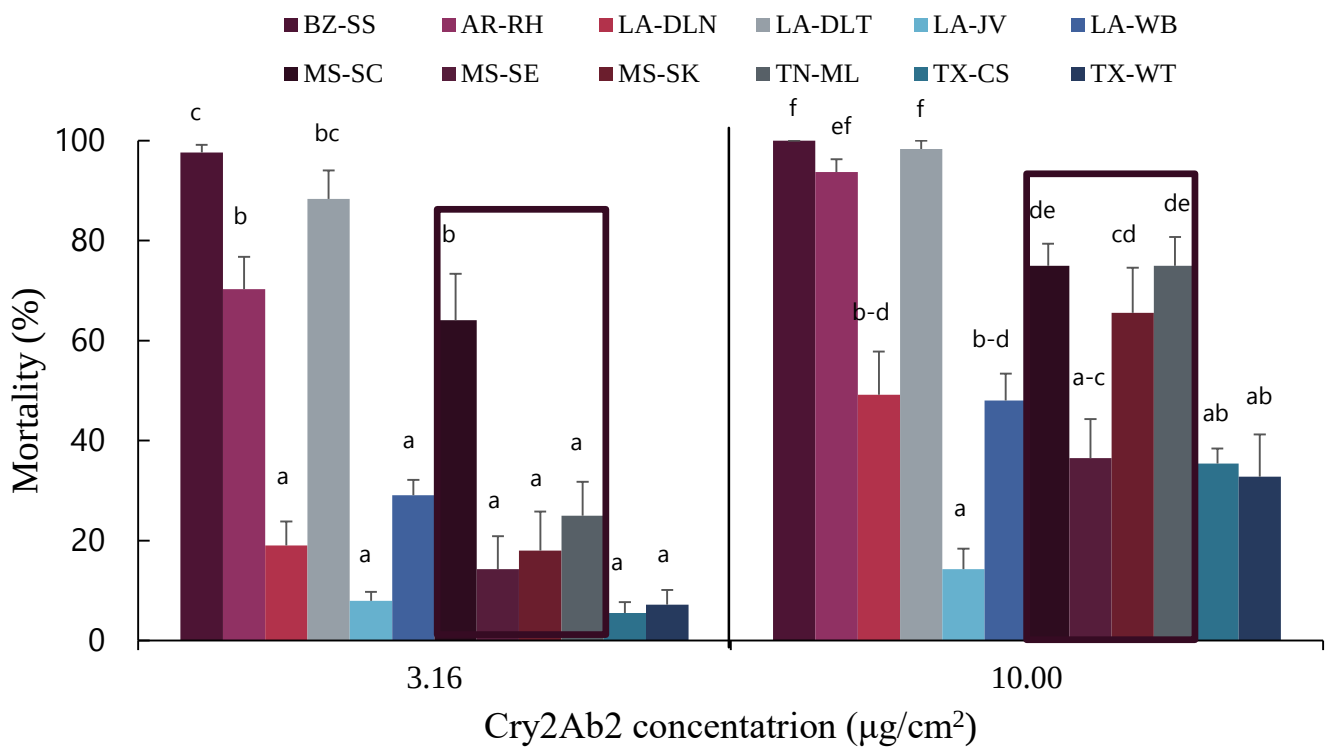


CRY1AC Comparing Populations

CRY2AB2 DATA - 2016

Insect strain	LC_{50}-1 (95% CL) ($\mu g/cm^2$)	RR-1	LC_{50}-2 (95% CL) ($\mu g/cm^2$)	RR-2
BZ-SS	0.13 (0.10, 0.17)	1.0	0.09 (0.07, 0.11)	1.0
LA-AD	6.03 (4.32, 8.59)	46.4 *	3.21 (2.19, 4.59)	35.7 *
TN-JN	17.34 (12.42, 26.71)	133.4*	12.00 (9.00, 16.55)	133.3*
TN-BG2	1.78 (1.35, 2.42)	13.7 *	0.36 (0.30, 0.43)	4.0
MS-LD	1.36 (0.94, 2.06)	10.5 *	0.77 (0.56, 1.07)	8.6
AR-TK	0.31 (0.21, 0.47)	2.4	0.09 (0.06, 0.12)	1.0

LC_{50} and 95% confidence limits (CL) based on larval mortality of *Helicoverpa zea* to Cry2Ab2 in 2017.								
Insect strain	Host	Traits	N[a]	LC_{50} (95% CL) (µg/cm^2)[b]	Slope ± SE	X^2	df	Resistance ratio[c]
BZ-SS	Laboratory	/	960	0.20 (0.17, 0.24)	1.61 ± 0.09	30.7	26	1.0
LA-WB-NBt corn	NBT-Corn	/	953	> 10.00	/	/	/	> 50.0 *
TX-CS-WS	Widestrike-Cotton	Cry1Ac, Cry1F	959	> 10.00	/	/	/	> 50.0 *
TX-Wharton-TL	Twinlink-Cotton	Cry1Ab, Cry2Ae	960	> 10.00	/	/	/	> 50.0 *
MS-Stoneville-VT2P	Double Pro-Corn	Cry1A.105, Cry2Ab2	512	> 10.00	/	/	/	> 50.0 *
LA-DL-NBt cotton	NBt-Cotton	/	512	> 10.00	/	/	/	> 50.0 *
MS-Starkville-VT2P	Double Pro-Corn	Cry1A.105, Cry2Ab2	512	9.22 (2.88, 9313)	1.31 ± 0.53	49.5	26	46.1 *
AR-Rohwer-GS	Grain sorghum	/	512	1.21 (0.72, 2.22)	1.19 ± 0.17	71.8	26	6.1
MS-Silver City-BG2	Bollgard 2-Cotton	Cry1Ac, Cry2Ab	512	2.28 (1.25, 4.94)	1.18 ± 0.20	66.7	26	11.4 *
LA-DL-TL	Twinlink-Cotton	Cry1Ab, Cry2Ae	512	0.65 (0.38, 1.54)	1.28 ± 0.18	69.3	26	3.3
LA-Jonesville-BG2	Bollgard 2-Cotton	Cry1Ac, Cry2Ab	512	> 10.00	/	/	/	> 50.0 *
MS-Benoit-BG2	Bollgard 2-Cotton	Cry1Ac, Cry2Ab	512	6.18 (3.53, 14.27)	1.05 ± 0.15	44.4	26	30.9 *
TN-Milan-VT2P	Double Pro-Corn	Cry1A.105, Cry2Ab2	960	0.20 (0.17, 0.24)	1.61 ± 0.09	30.7	26	1.0
TN-Jackson-Obsession II	Obsession II-Sweet corn	Cry1A.105, Cry2Ab2	512	6.46 (4.05, 12.08)	0.91 ± 0.10	25	26	32.3*



CRY2AB2 Comparing Populations

VIP3A DATA - 2016

Insect strain	LC_{50}-1 (95% CL) ($\mu g/cm^2$)	RR-1	LC_{50}-2 (95% CL) ($\mu g/cm^2$)	RR-2
BZ-SS	0.97 (0.85, 1.11)	1.0	0.82 (0.69, 0.97)	1.0
LA-AD	0.19 (0.15, 0.24)	-5.1	0.12 (0.10, 0.14)	-6.8
TN-JN	0.16 (0.12, 0.21)	-6.1	0.13 (0.09, 0.17)	-6.3
TN-BG2	0.18 (0.13, 0.23)	-5.4	0.12 (0.09, 0.16)	-6.8
MS-LD	0.14 (0.12, 0.16)	-6.9	0.11 (0.09, 0.12)	-7.5
AR-TK	0.17 (0.13, 0.23)	-5.7	0.13 (0.10, 0.17)	-6.3

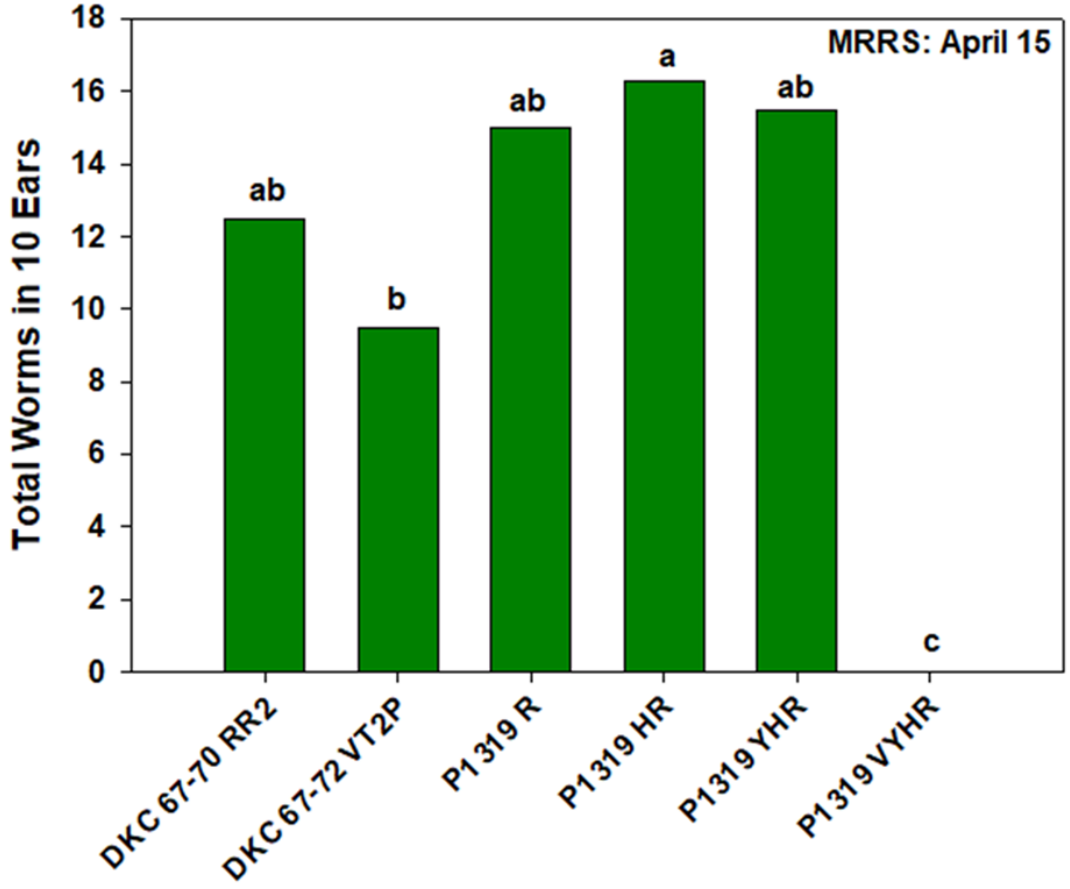
LC_{50} and 95% confidence limits (CL) based on larval mortality of *Helicoverpa zea* to Vip3A in 2017.								
Insect strain	Host	Traits	N[a]	LC_{50} (95% CL) (μg/cm^2)[b]	Slope ± SE	X^2	df	Resistance ratio[c]
BZ-SS	Laboratory	/	895	0.96 (0.86, 1.12)	2.81 ± 0.28	22.2	22	1.0
LA-WB-NBt corn	NBT-Corn	/	895	0.33 (0.18, 0.61)	2.08 ± 0.43	75.0	22	-2.9
TX-CS-WS	Widestrike-Cotton	Cry1Ac, Cry1F	896	0.03 (0.02, 0.04)	2.08 ± 0.43	75.0	22	-32.0
TX-Wharton-TL	Twinlink-Cotton	Cry1Ab, Cry2Ae	895	0.82 (0.73, 0.90)	5.67 ± 0.87	11.0	22	-1.2
MS-Stoneville-VT2P	Double Pro-Corn	Cry1A.105, Cry2Ab2	894	0.06 (0.04, 0.07)	1.84 ± 0.18	48.0	22	-16.0
LA-DL-NBt cotton	NBt-Cotton	/	448	0.10 (0.08, 0.12)	2.37 ± 0.22	10.9	22	-9.6
MS-Starkville-VT2P	Double Pro-Corn	Cry1A.105, Cry2Ab2	448	0.04 (0.03, 0.05)	2.62 ± 0.27	9.6	22	-24.0
AR-Rohwer-GS	Grain sorghum	/	448	1.25 (1.00, 1.57)	5.24 ± 1.23	38.8	22	1.3
MS-Silver City-BG2	Bollgard 2-Cotton	Cry1Ac, Cry2Ab	448	0.05 (0.04, 0.06)	3.20 ± 0.34	15.2	22	-19.2
LA-DL-TL	Twinlink-Cotton	Cry1Ab, Cry2Ae	448	0.33 (0.19, 0.61)	1.70 ± 0.31	15.2	22	-2.9
LA-Jonesville-BG2	Bollgard 2-Cotton	Cry1Ac, Cry2Ab	448	0.15 (0.12, 0.18)	2.05 ± 0.18	18.7	22	-6.4
MS-Benoit-BG2	Bollgard 2-Cotton	Cry1Ac, Cry2Ab	448	0.04 (0.03, 0.06)	1.29 ± 0.15	35.2	22	-24.0
TN-Milan-VT2P	Double Pro-Corn	Cry1A.105, Cry2Ab2	448	0.12 (0.10, 0.14)	2.30 ± 0.21	22.3	22	-8.0

BIOASSAY DATA SUGGESTS

- "Field-evolved (or field-selected) resistance as a genetically based decrease in susceptibility of a population to a toxin caused by exposure of the population to the toxin in the field"
  - Cry1Ac
    - Every field-collected population, 100%, assayed in 2017 exhibited >10-fold resistance ratio to Cry1Ac
  - Cry2Ab2
    - 75% of the field-collected populations assayed in 2017 exhibited >10-fold resistance ratio to Cry2ab2
    - 69% of the field-collected populations assayed in 2017 exhibited >30-fold resistance ratio to Cry2Ab2
  - Vip3A – no resistance detected
  - "Resistance ratios >10 are more likely to reflect genetically based decreases in susceptibility"
    - There has been a genetic shift for decreased susceptibility to Cry1Ac and Cry2Ab2

WHAT IS DRIVING BT RESISTANCE?

- We theorize that bollworms/corn earworms are being selected for resistance to Bt technologies in corn
- Progeny from populations developed in corn infest cotton
- So what does this mean for Vip3A?

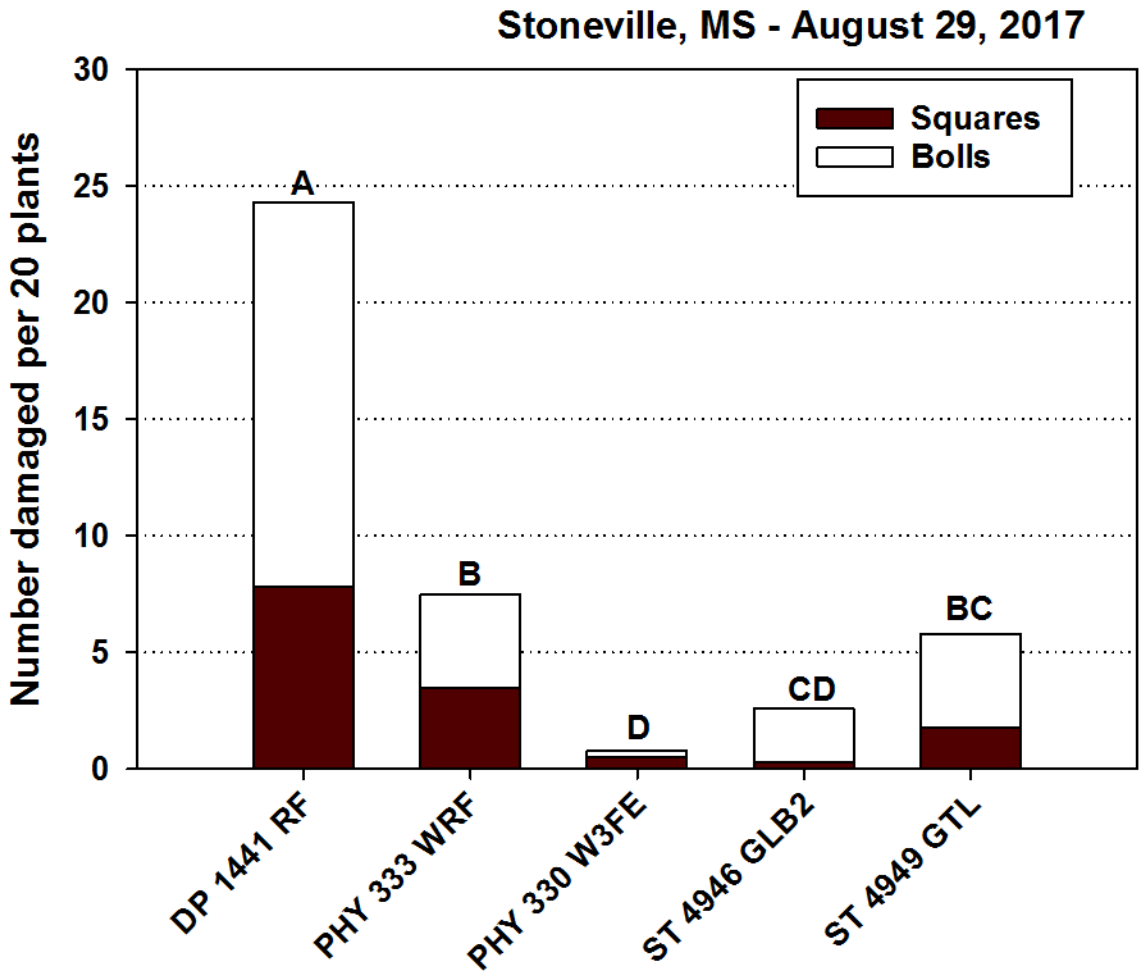


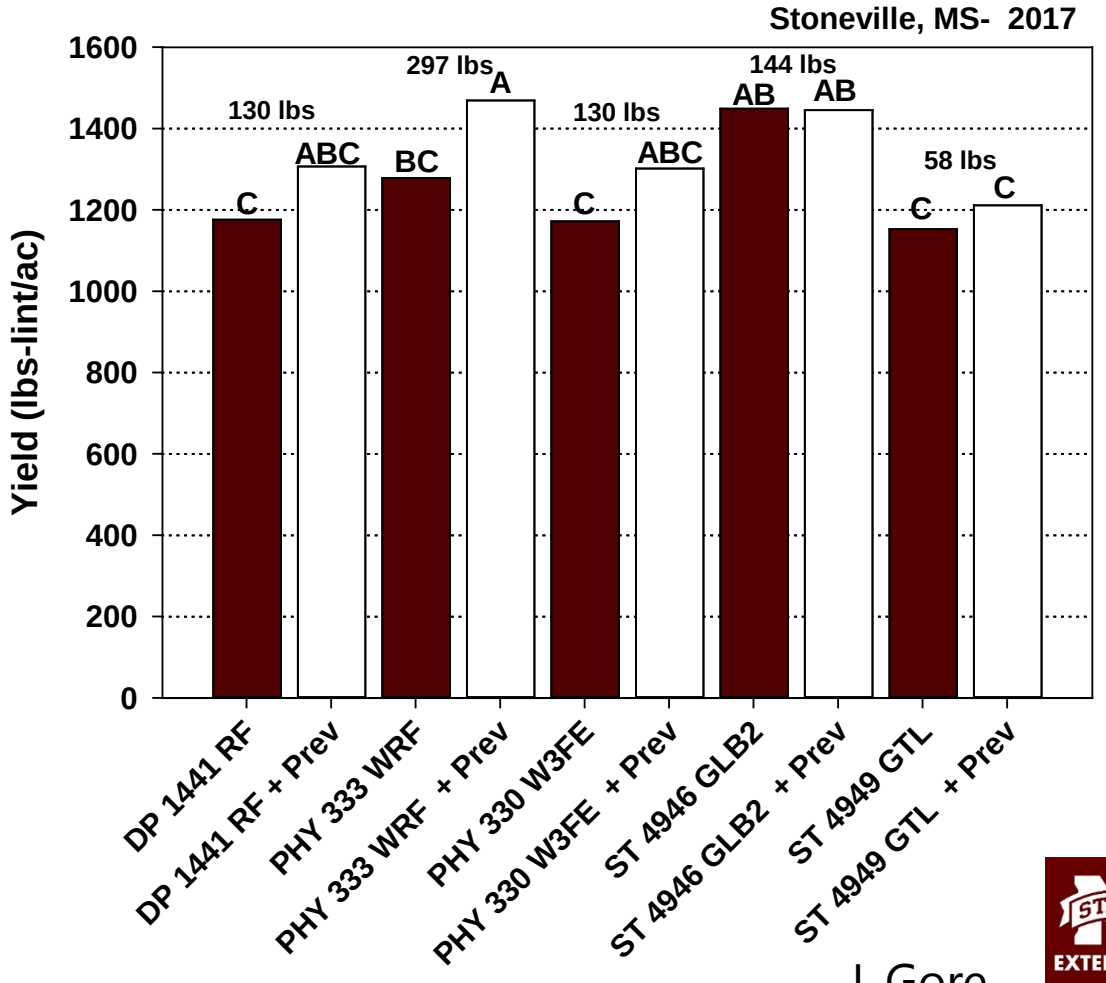
A. Catchot

FIELD PERFORMANCE

EFFICACY OF BT COTTON TECHNOLOGIES AND VALUE OF TREATING WITH INSECTICIDE

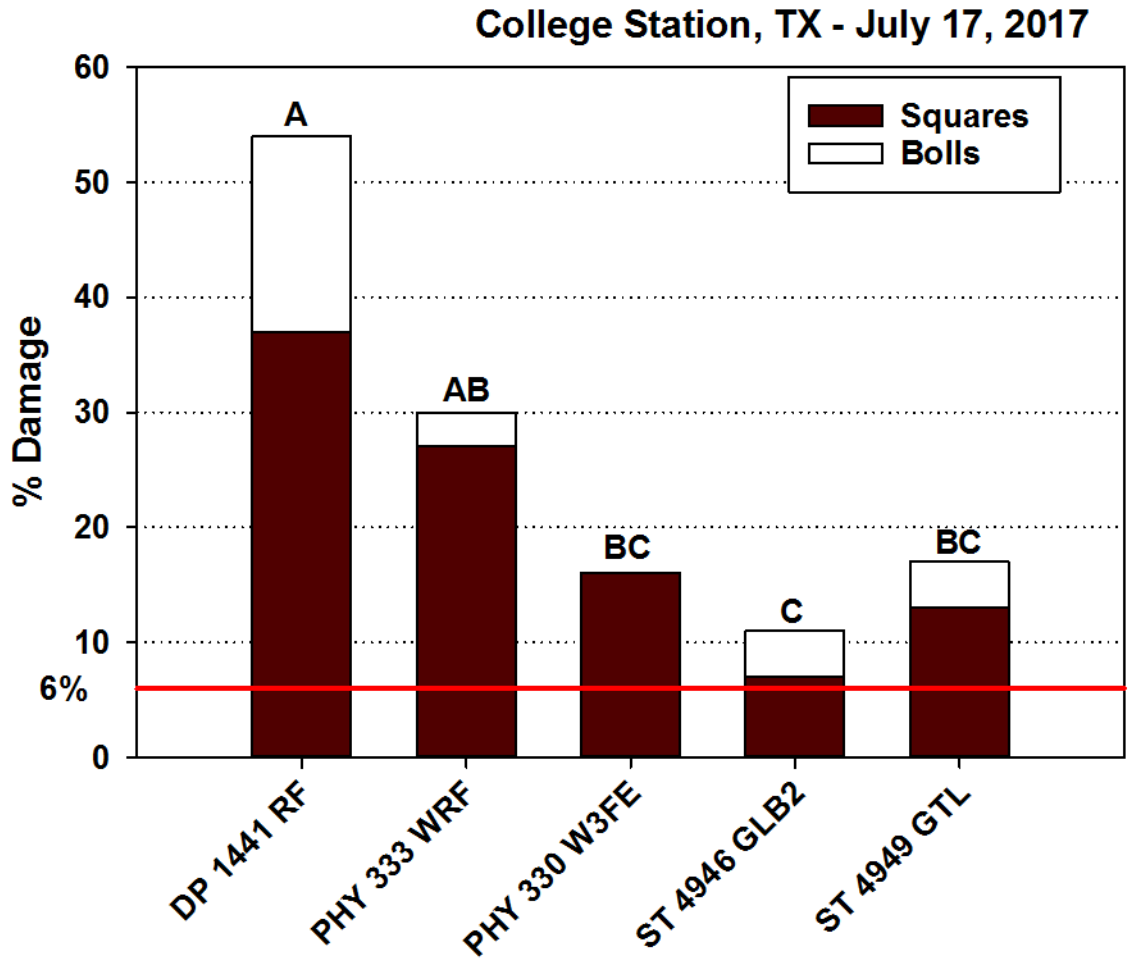
BOLLWORM INJURY TO BT COTTON – STONEVILLE, MS

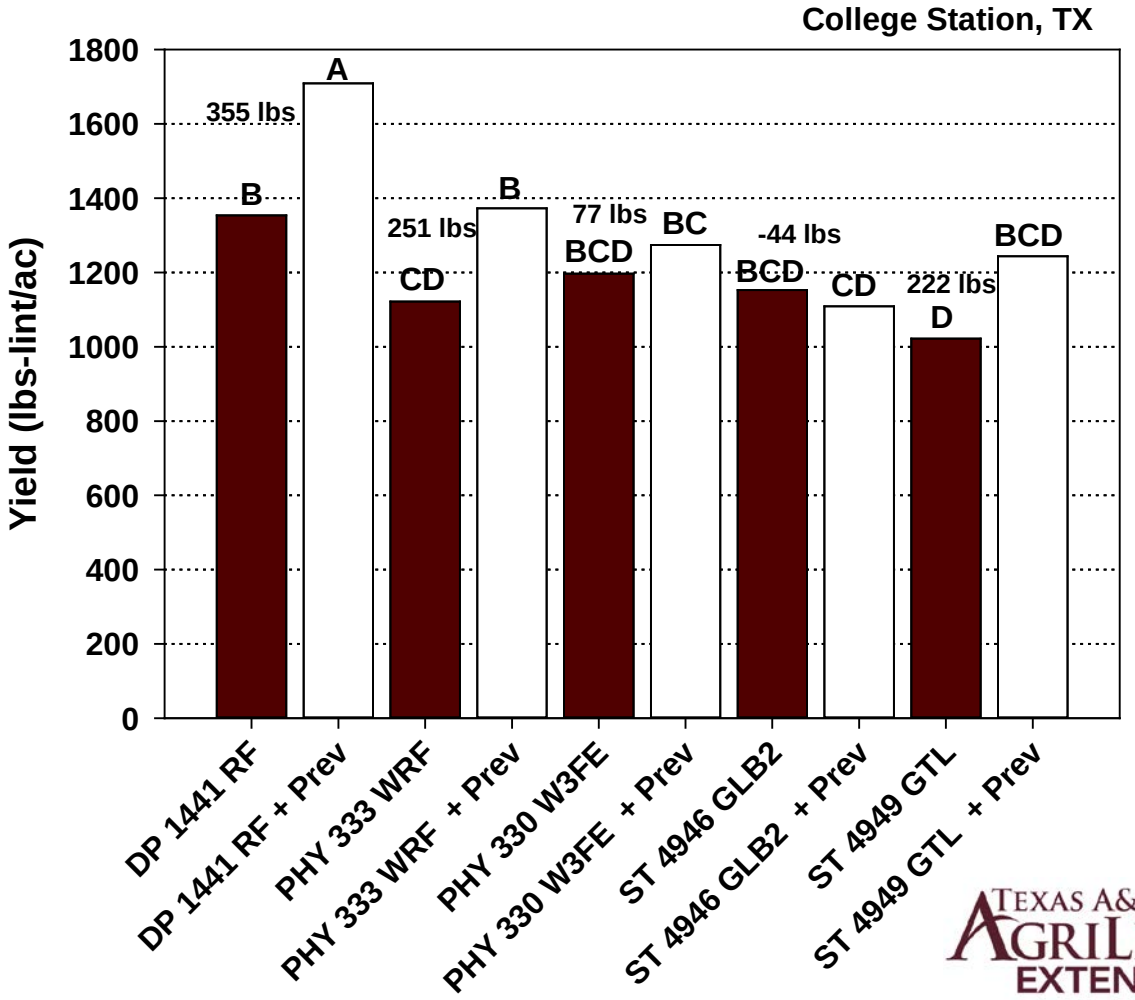




J. Gore

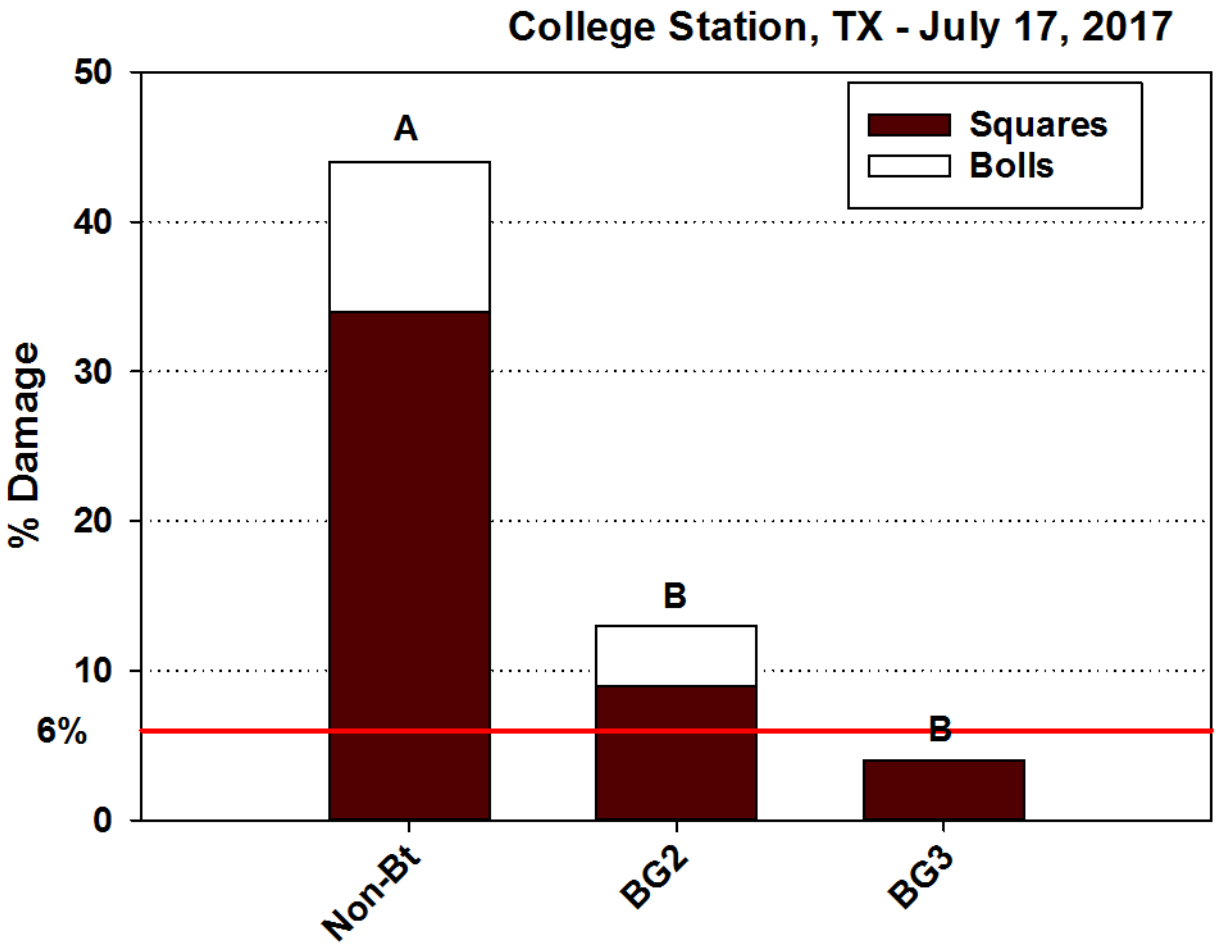
BOLLWORM INJURY TO BT COTTON – COLLEGE STATION, TX

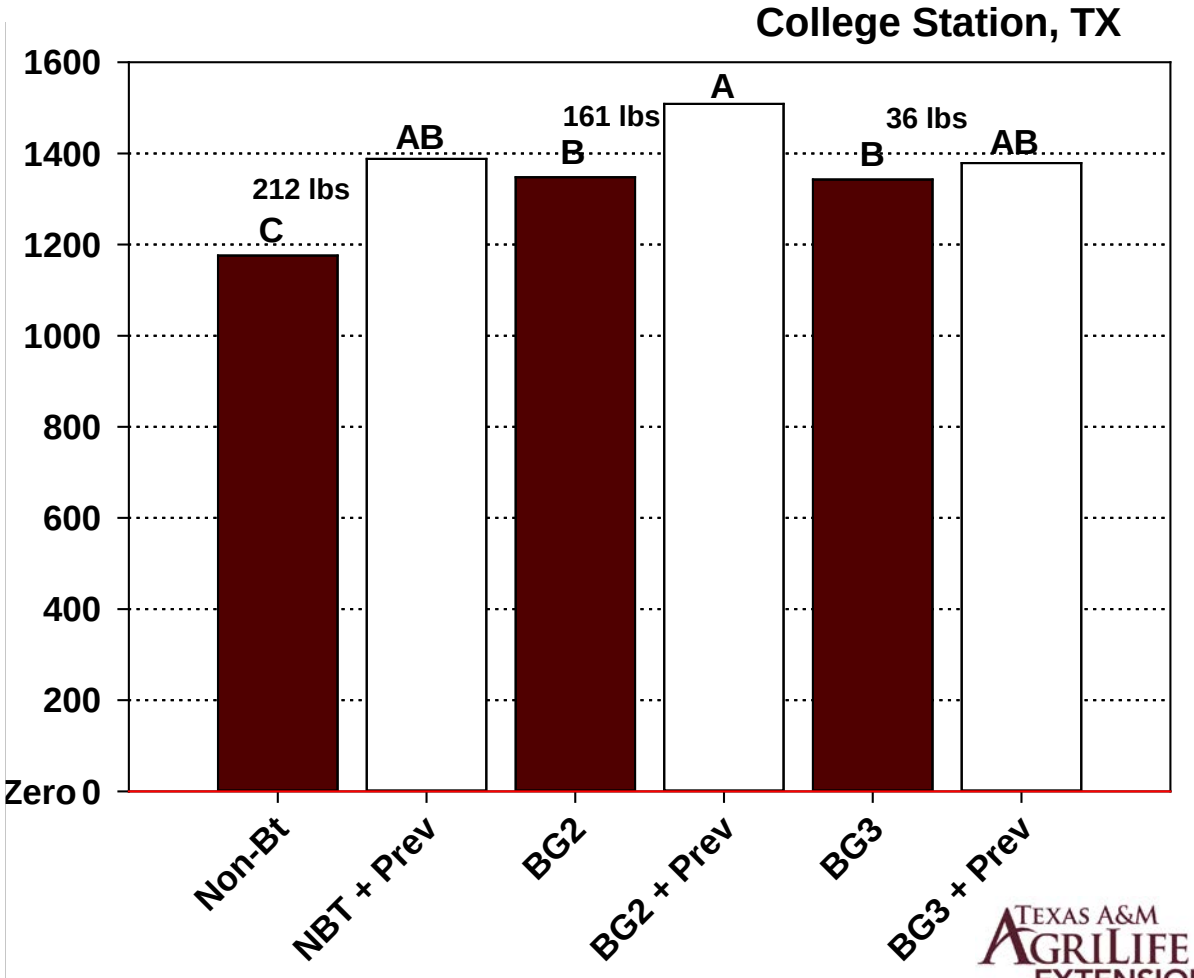




TEXAS A&M AGRILIFE EXTENSION

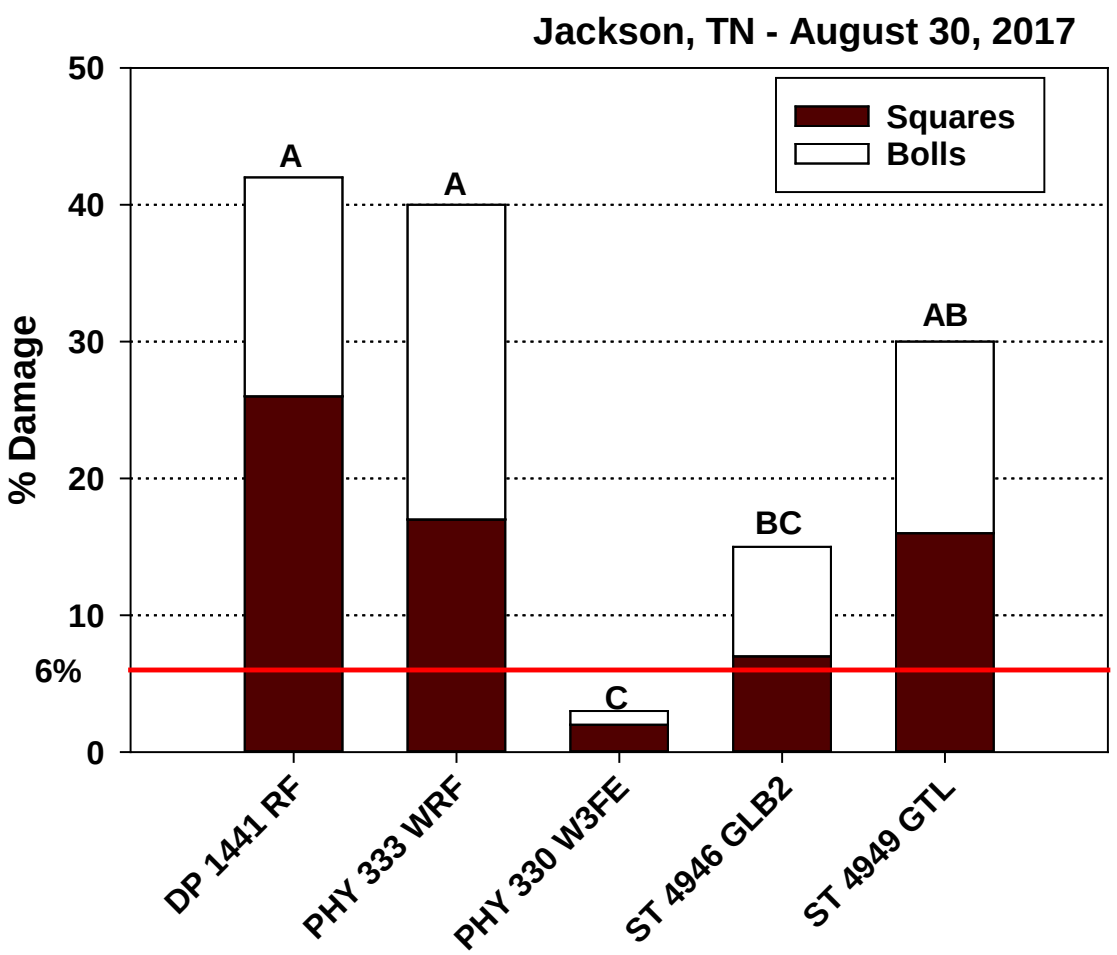
PERFORMANCE OF BOLLGARD 3 – COLLEGE STATION, TX

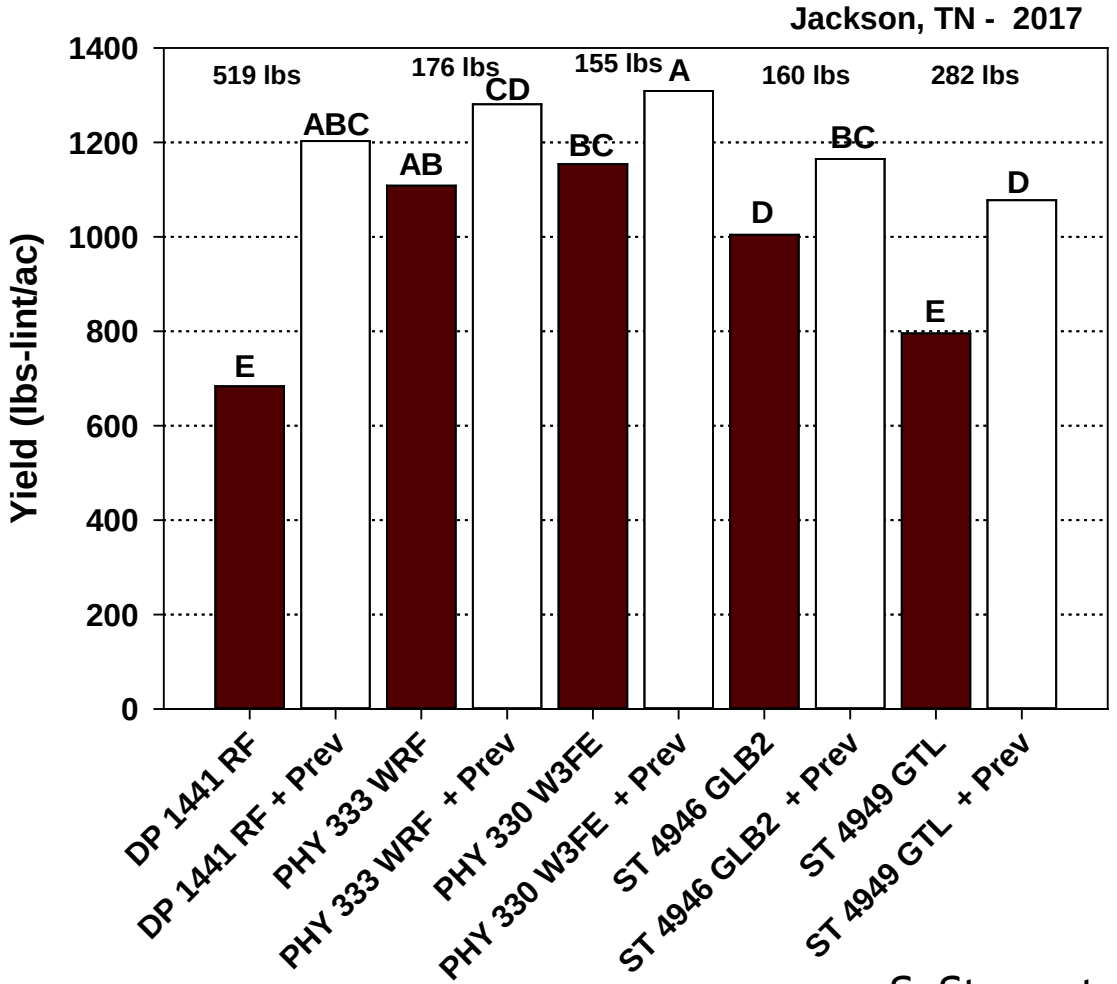




TEXAS A&M AGRILIFE EXTENSION

BOLLWORM INJURY TO BT COTTON – JACKSON, TN



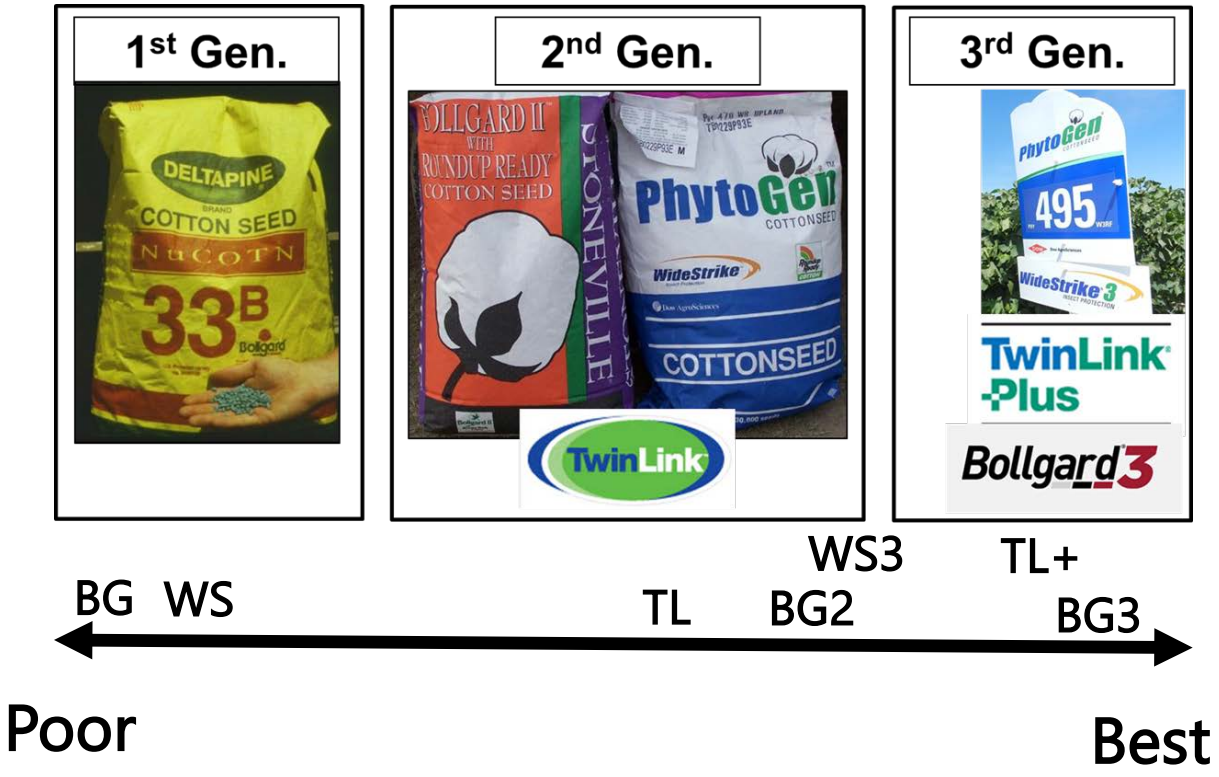


S. Stewart

Extension

SUMMARY

- Cry1F is ineffective
- Cry1Ac and Cry1Ab bring very little to the table for control of bollworm
  - High resistance
- Cry2Ab and Cry2Ae are providing low to moderate efficacy
  - Moderate to high resistance
- Vip3A provides moderate efficacy
- Cry1s + Cry2s + Vip pyramided provide moderate to high efficacy
- Keep paying for more for new technology that is not providing better control than old technologies in the past
- Sustainability of Vip is questionable?



QUESTIONS?

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United States Department of Agriculture
National Institute of Food and Agriculture