# Bt Resistance

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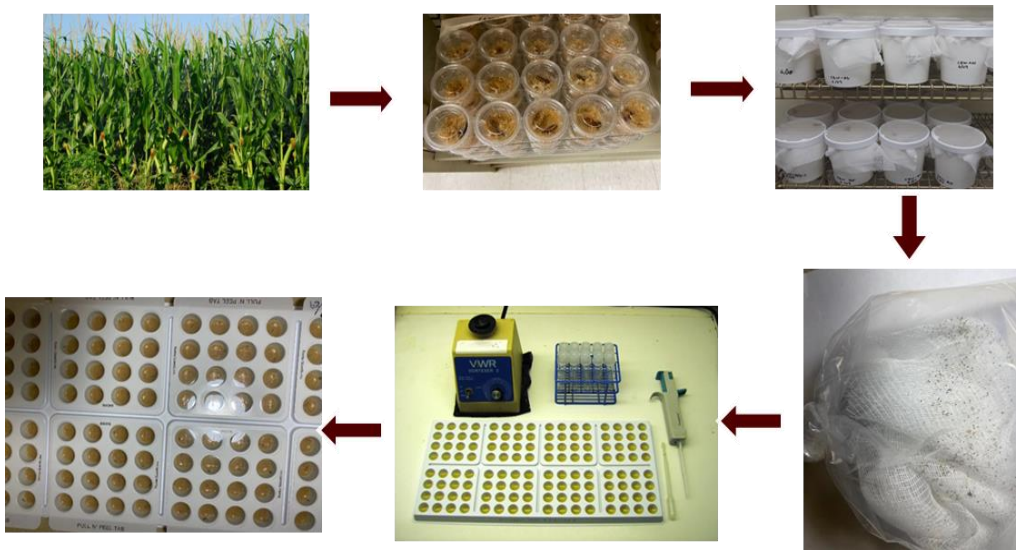
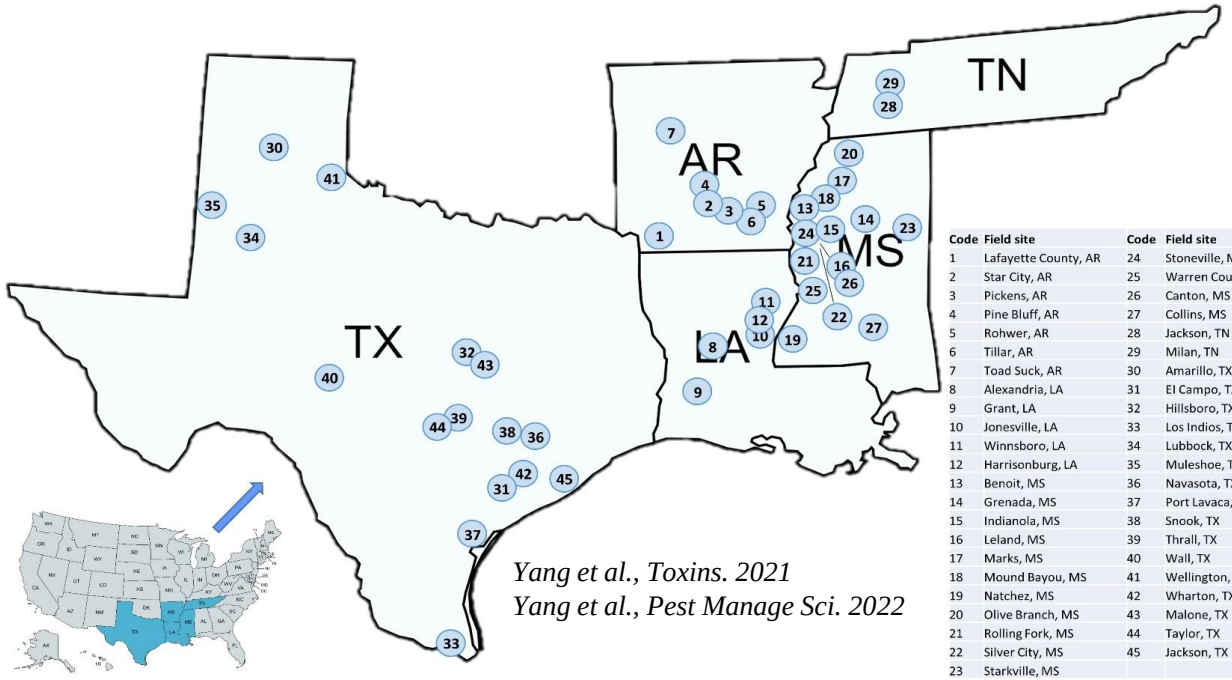
# Bt Proteins Used in Bt Corn and Cotton

| Crop | Cry1 | Cry2 | Vip3A |
|---|---|---|---|
| Cotton | Cry1Ac, Cry1Ab, Cry1F | Cry2Ab, Cry2Ae | Vip3A19 |
| Corn | Cry1Ab, Cry1F, Cry1A.105 | Cry2Ab2 | Vip3A20 |

# Corn Earworm Bt Resistance Monitoring (Cry1Ac, Cry2Ab2, Vip3Aa)





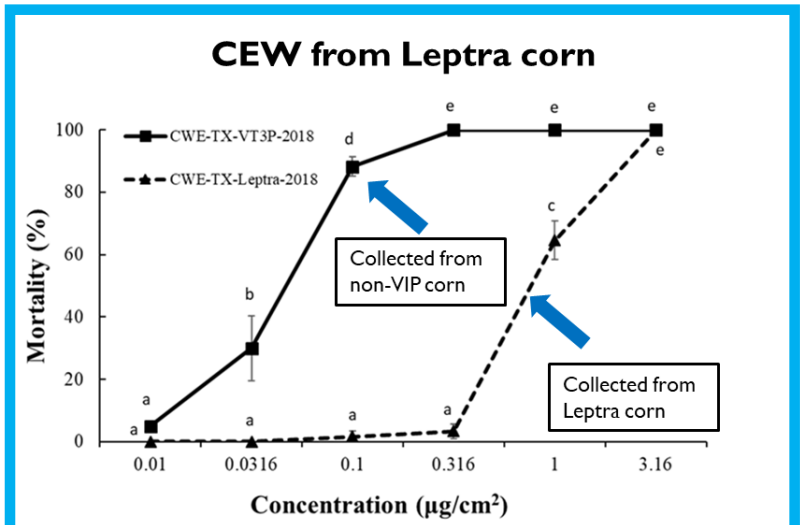

| Code | Field site | Code | Field site |
|---|---|---|---|
| 1 | Lafayette County, AR | 24 | Stoneville, MS |
| 2 | Star City, AR | 25 | Warren County, MS |
| 3 | Pickens, AR | 26 | Canton, MS |
| 4 | Pine Bluff, AR | 27 | Collins, MS |
| 5 | Rohwer, AR | 28 | Jackson, TN |
| 6 | Tillar, AR | 29 | Milan, TN |
| 7 | Toad Suck, AR | 30 | Amarillo, TX |
| 8 | Alexandria, LA | 31 | El Campo, TX |
| 9 | Grant, LA | 32 | Hillsboro, TX |
| 10 | Jonesville, LA | 33 | Los Indios, TX |
| 11 | Winnsboro, LA | 34 | Lubbock, TX |
| 12 | Harrisonburg, LA | 35 | Muleshoe, TX |
| 13 | Benoit, MS | 36 | Navasota, TX |
| 14 | Grenada, MS | 37 | Port Lavaca, TX |
| 15 | Indianola, MS | 38 | Snook, TX |
| 16 | Leland, MS | 39 | Thrall, TX |
| 17 | Marks, MS | 40 | Wall, TX |
| 18 | Mound Bayou, MS | 41 | Wellington, TX |
| 19 | Natchez, MS | 42 | Wharton, TX |
| 20 | Olive Branch, MS | 43 | Malone, TX |
| 21 | Rolling Fork, MS | 44 | Taylor, TX |
| 22 | Silver City, MS | 45 | Jackson, TX |
| 23 | Starkville, MS | | |

*Yang et al., Toxins. 2021*
*Yang et al., Pest Manage Sci. 2022*

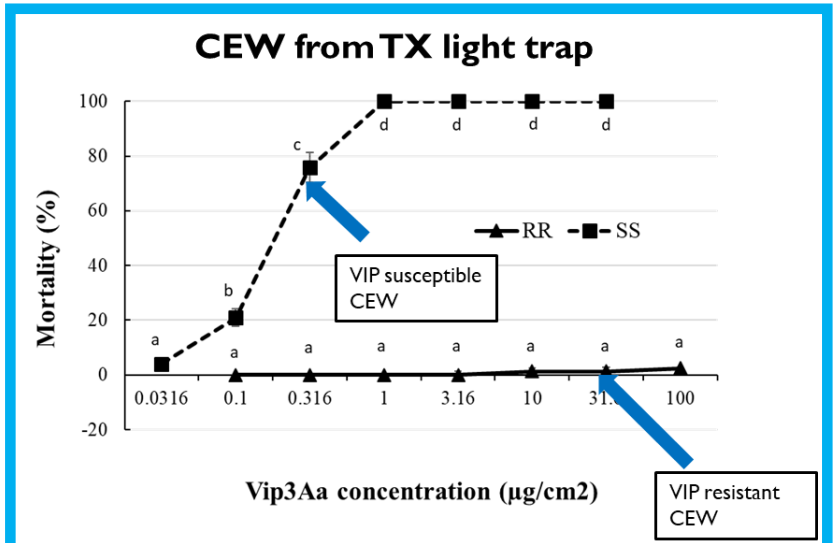
**174 field populations with >267,264 insects from 2016-2023**

# Diet-overlay Bioassays (2016-2023)

| *Bt* protein | Percentage of populations with RR > 10X | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2016 (5) | 2017 (14) | 2018 (34) | 2019 (30) | 2020 (5) | 2021 (12) | 2022 (37) | 2023 (37) |
| Cry1Ac | / | 100% | 94% | 96% | 100% | 92% | 100% | 100% |
| Cry2Ab2 | 80% | 77% | 73% | 73% | 100% | 92% | 74% | 97% |
| Vip3Aa | 0% | 0% | 0%* | 0%* | 0% | 0% | 0% | 0% |

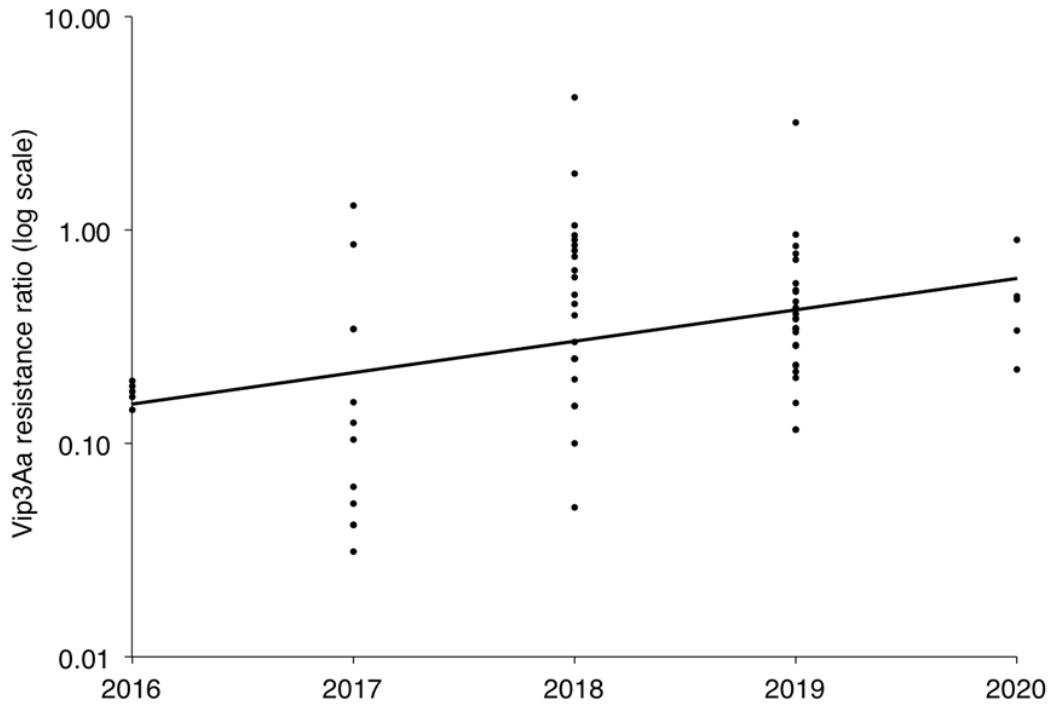



Low level of resistance
(probably minor genes?)




Complete resistance
(major gene controlled)

# Early Warning of Resistance to Vip3Aa Protein




Open Access Article

## Early Warning of Resistance to Bt Toxin Vip3Aa in *Helicoverpa zea*

by Fei Yang [1,*], David L. Kerns [1], Nathan S. Little [2], José C. Santiago González [1] and Bruce E. Tabashnik [3,*]

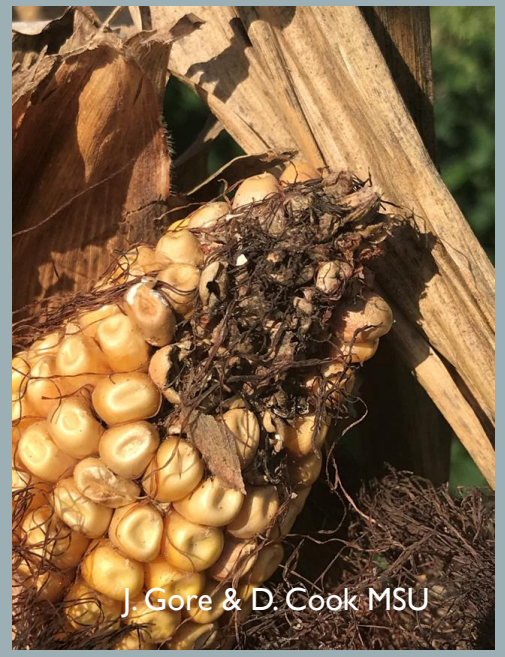
[1] Department of Entomology, Texas A&M University, College Station, TX 77843, USA
[2] USDA Agricultural Research Service, Stoneville, MS 38776, USA
[3] Department of Entomology, University of Arizona, Tucson, AZ 85721, USA
* Authors to whom correspondence should be addressed.

*Toxins* **2021**, *13*(9), 618; https://doi.org/10.3390/toxins13090618

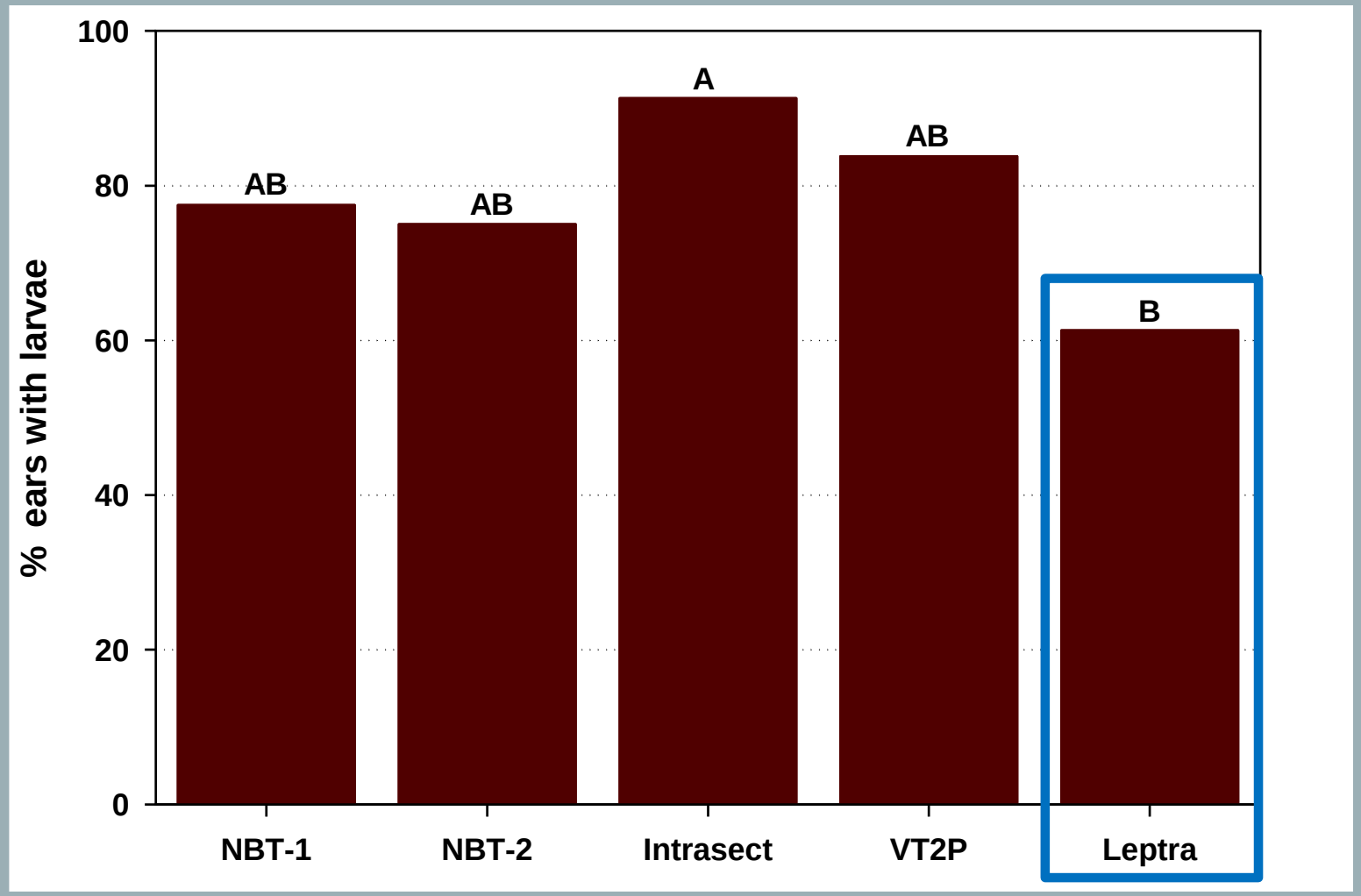
Increase from 2016 to 2020 in the Vip3Aa resistance ratio relative to the BZ lab strain for 71 field-derived strains of CEW. Linear regression: log (y) = 0.14X – 282, $R^2$ = 0.12, df = 69, P = 0.003.

# CORN EARWORM FEEDING IN LEPTRA CORN TX & MS -2018-19






# 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 |

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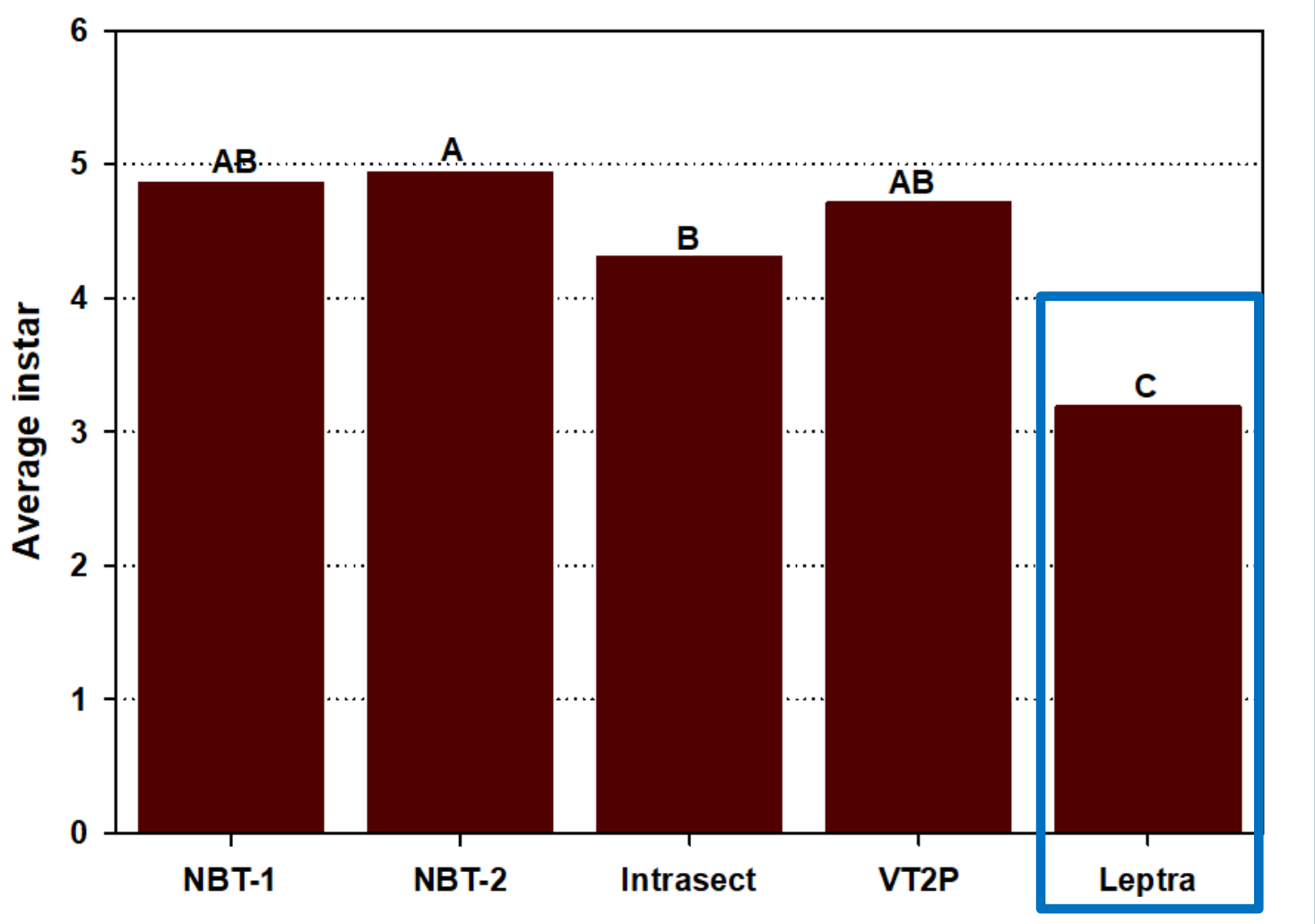


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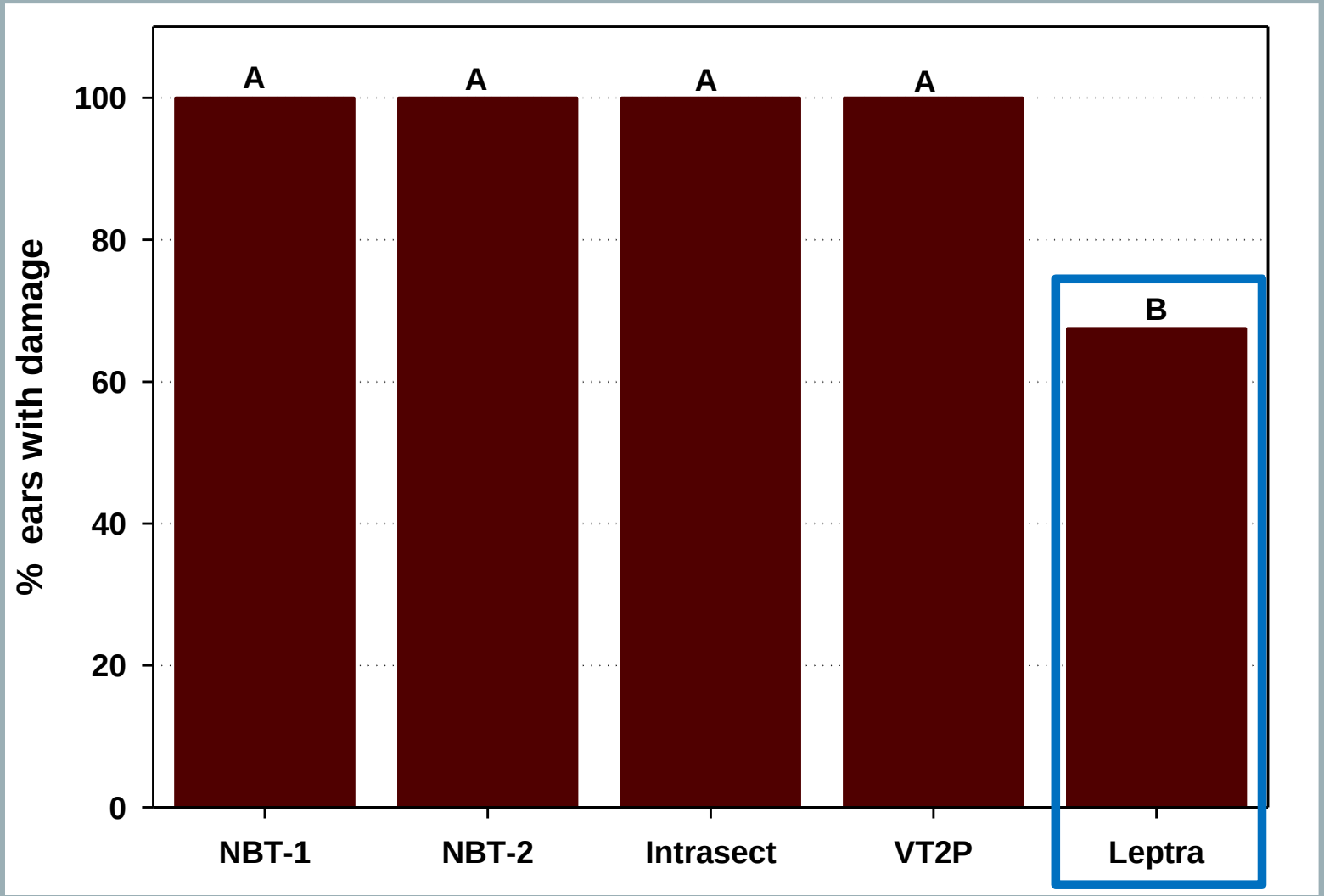


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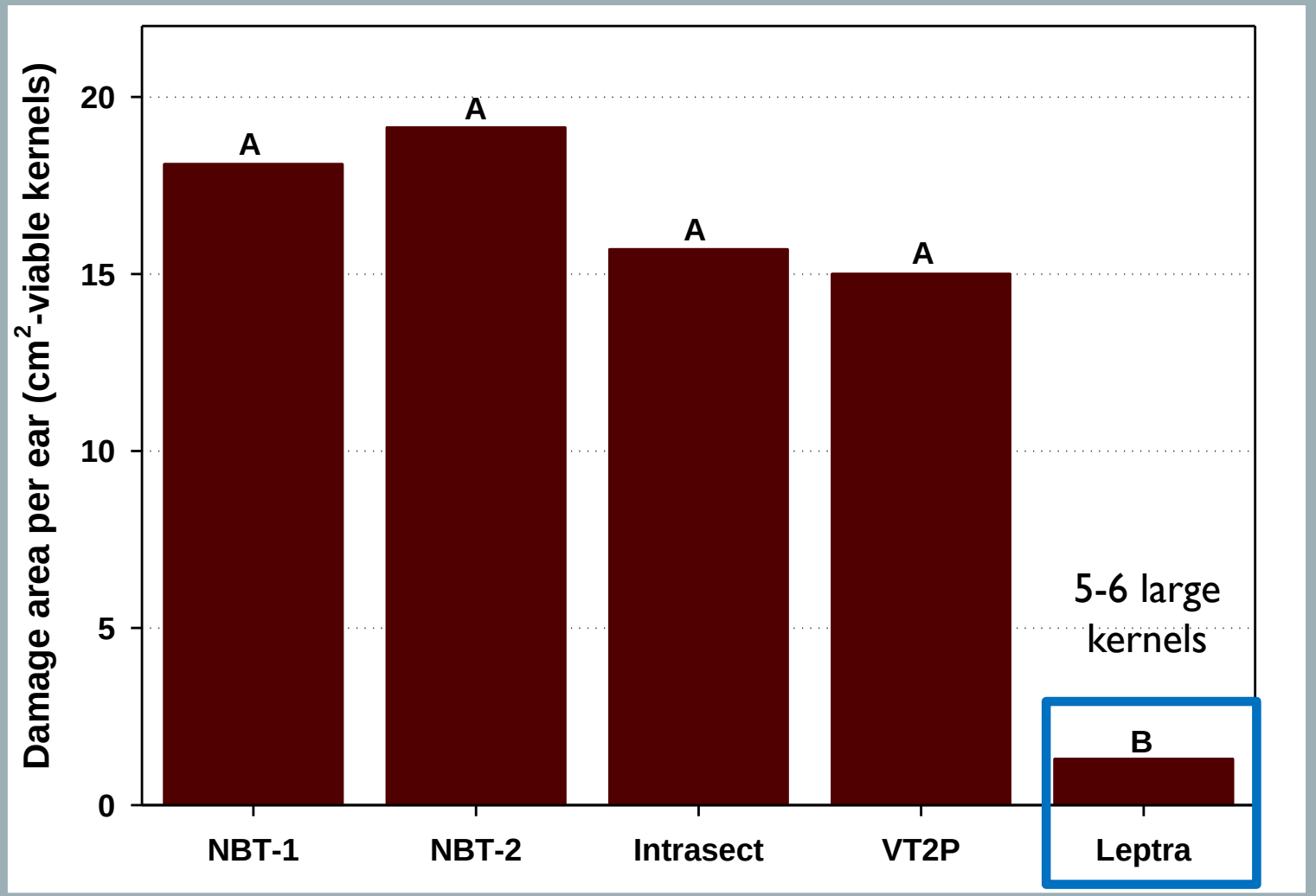


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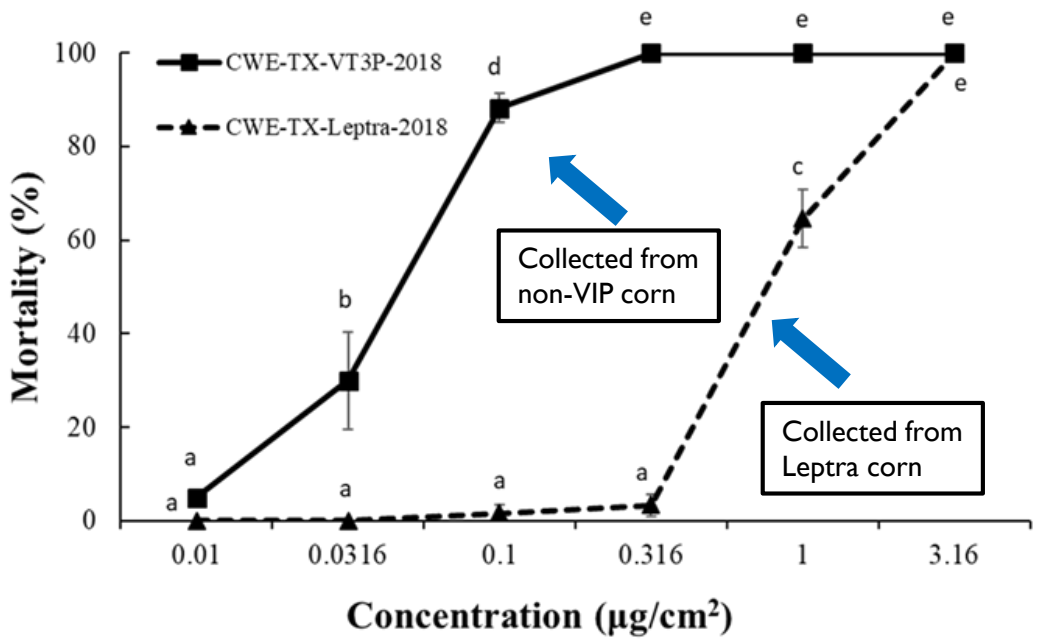
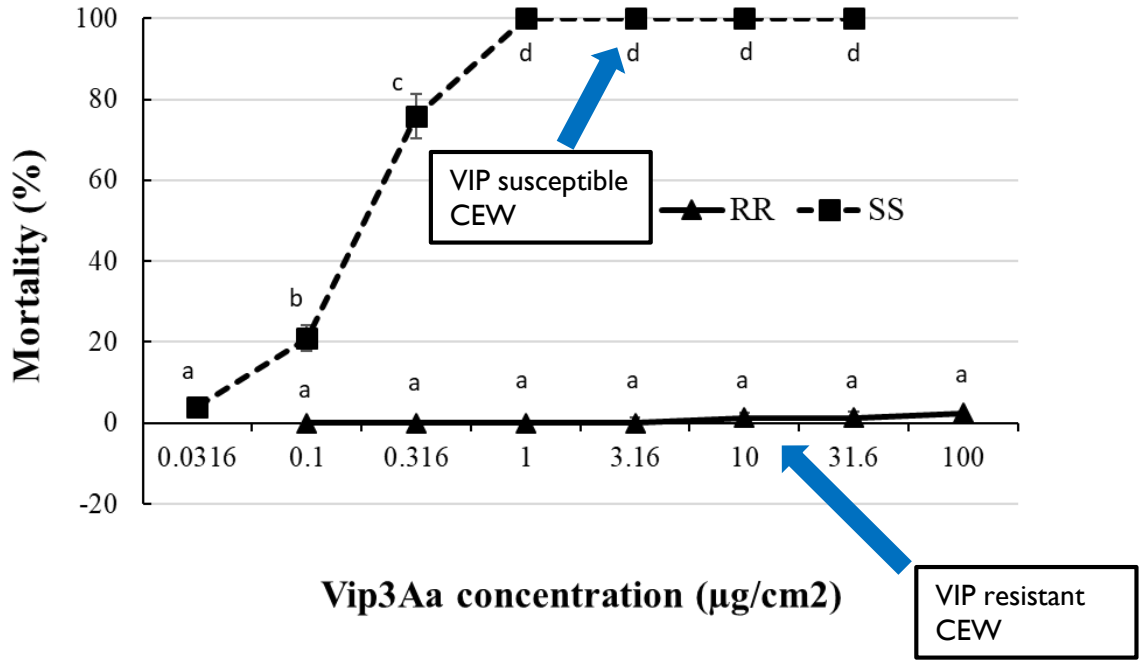
# Two Types of Vip3Aa Resistance?

## CEW FROM LEPTRA CORN




Low level of resistance
(probably minor genes)

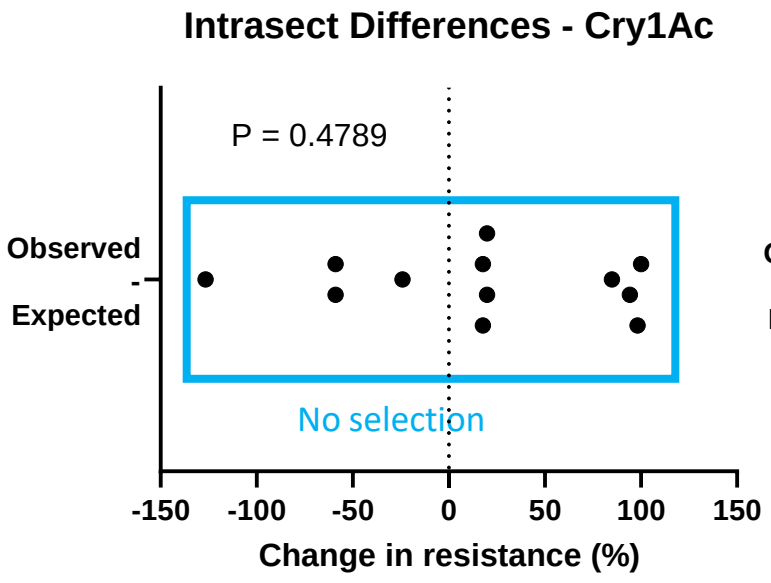
## CEW FROM TX LIGHT TRAP

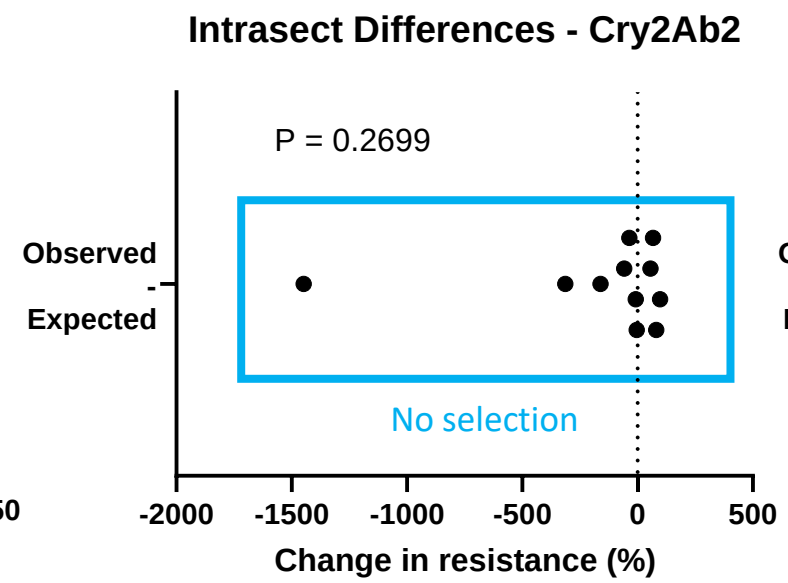


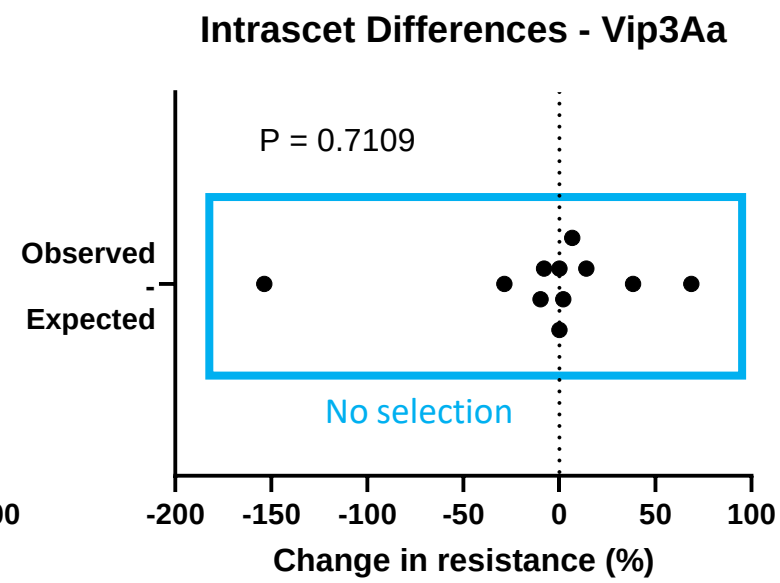

Complete resistance
(major gene controlled)

# Bt Resistance Selection Pressure from Intrasect

$$\%\ \boldsymbol{change\ in\ susceptibility} = \left(1 - \left(\frac{(\text{susceptible strain } LC_{50} \times \text{non-Bt collection } LC_{50})}{(\text{susceptible strain } LC_{50} \times \text{Bt hybrid collection } LC_{50})}\right)\right) \times 100$$



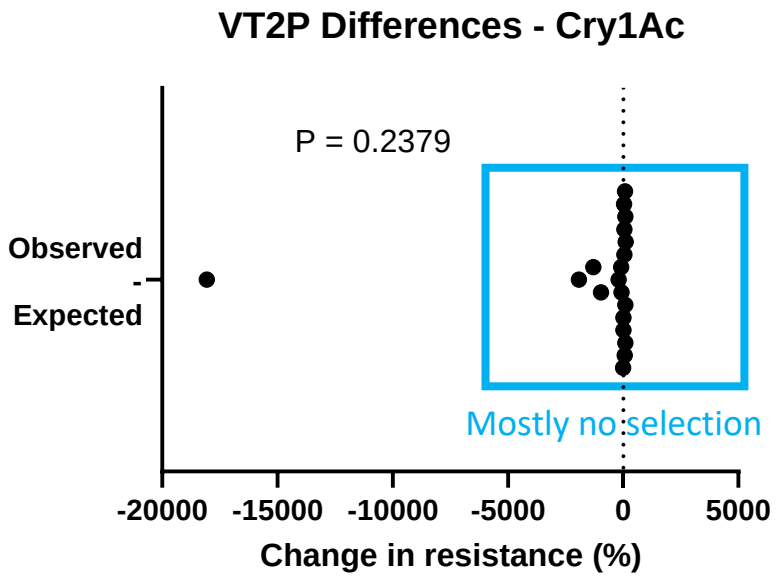



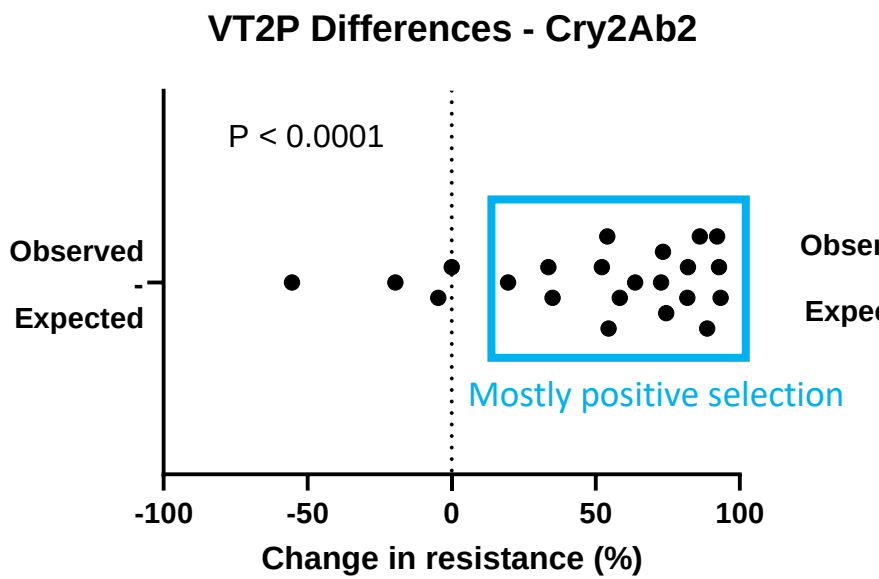



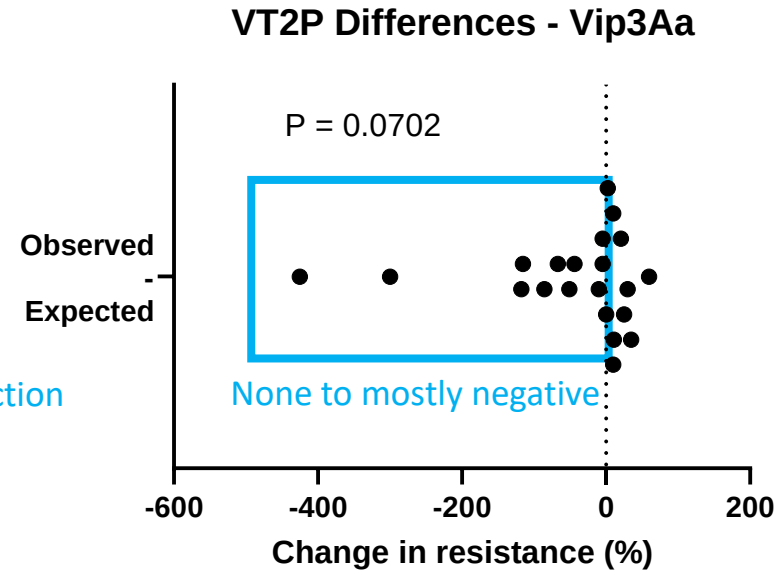

Intrasect = Cry1Ab + Cry1F

# Bt Resistance Selection Pressure from VT2P

$$\%\ \boldsymbol{change\ in\ susceptibility} = \left(1 - \left(\frac{(\text{susceptible strain } LC_{50} \times \text{non–Bt collection } LC_{50})}{(\text{susceptible strain } LC_{50} \times \text{Bt hybrid collection } LC_{50})}\right)\right) \times 100$$




VT2P = Cry1A.105 + Cry2Ab2

# *H. zea*-$F_2$ FAMILIES SURVIVING THE DIAGNOSTIC CONCENTRATION OF VIP3Aa39 (3µg/cm$^2$)

| Collection site | Year of collection | Method to establish the $F_2$ families | Number of $F_2$ families screened | Number of surviving $F_2$ families[1,2] | Estimated number of resistance alleles[3] | Estimated resistance allele frequency | Confidence Interval (95%) |
|---|---|---|---|---|---|---|---|
| **Texas** | **2018-2019[1]** | Cross with SS♂ Light trap | 126 | 2 **(1.59%)** | 2 | 0.0042 | (0.0011 – 0.0151) |
| **Midsouth** | **2019-2020[2]** | Cross with SS♀ | 192 | 5 **(2.60%)** | 5 | 0.0130 | (0.0056-0.0301) |
| **Overall** | **2018-2020** | Cross with SS♂ Light trap Cross with SS♀ | 318 | 7 **(2.20%)** | 7 | 0.0081 | (0.0039-0.0166) |

1. Total insects assayed in 2018 and 2019 = 16,128 larvae
2. Total insects assayed 2019 and 2020 = 24,576 larvae
3. Based on 128 larvae per bioassay/$F_2$ family
4. 5 survivors ≥ 2nd instar with at least 1 larva ≥ 3rd instar
5. Number of resistant alleles based on results from simple monogenic inheritance models ($\chi^2 < 3.841$ with 1 df, $p > 0.05$)

Jose Santiago Gonzalez, PhD student

# VIP3Aa RESISTANT STRAINS IDENTIFIED

| Insect Family | Origin | Host | N | No. survivors | No. insects within instar | | |
|---|---|---|---|---|---|---|---|
| | | | | | 2nd instar | 3rd instar | 4th instar |
| LA-M1 | Alexandria, LA | BG2 Cotton | 128 | 21 | 0 | 4 | 17 |
| LA-AC4 | Winnsboro, LA | VT2P Corn | 128 | 20 | 0 | 6 | 14 |
| MS-R2 | Stoneville, MS | Cry1Ab Sweet corn | 128 | 2 | 1 | 0 | 1 |
| MS-R15 | Stoneville, MS | Cry1Ab Sweet corn | 128 | 22 | 3 | 19 | 0 |
| MS-R21 | Stoneville, MS | VT2P Corn | 128 | 1 | 0 | 0 | 1 |

*Survivorship when exposed to 3.0 ug/cm$^2$ Vip3Aa39 diet overlays*

In 2019-2020 we identified 5 - F2 families carrying Vip3Aa resistant alleles

# VIP3Aa RESISTANT ALLELES

| Insect Family | Origin | Host | N | Observed survivors | Expected survivors for 2 alleles | $\chi^2$ | *P*-value |
|---|---|---|---|---|---|---|---|
| LA-M1 | Alexandria, LA | BG2 Cotton | 128 | 22.9 | 32 | 3.441 | 0.064 |
| LA-AC4 | Winnsboro, LA | VT2P Corn | 128 | 22.5 | 32 | 3.738 | 0.054 |
| MS-R15 | Stoneville, MS | Cry1Ab Sweet corn | 128 | 24.8 | 32 | 2.141 | 0.143 |

*Survivorship when exposed to 3.0 ug/cm$^2$ Vip3Aa39 diet overlays*

*P*-values > 0.05; indicated that these families were carrying 2 resistant alleles and were homozygous resistant for Vip3Aa

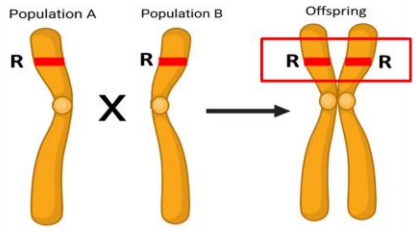
The collection from the Cry1Ab sweet corn in Stoneville, MS yielded - 3 Vip3Aa resistant alleles (RR, RS)

# Vip3Aa Resistant Populations

| Insect population | Collected location (Year) | LC50 (95% CL) (µg/cm$^2$) | Resistance ratio | Inheritance |
|---|---|---|---|---|
| CBW-BZ-SS | / | 0.11 (0.09, 0.13) | 1 | / |
| CBW-TX-VIP-RR | Snook, TX (2019) | > 100 | > 909.1 | Recessive, Autosomal, single-gene |
| CBW-LA-M1-VIP-RR | Alexandria, LA (2019) | > 100 | > 909.1 | Recessive, Autosomal, single-gene |
| CBW-MS-R2-VIP-RR | Stoneville, MS (2020) | > 100 | > 909.1 | Recessive, Autosomal, single-gene |
| CBW-MS-R15-VIP-RR | Stoneville, MS (2020) | > 100 | > 909.1 | Recessive, Autosomal, single-gene |
| CBW-LA-AC4-VIP-RR | Winnsboro, LA (2020) | > 100 | > 909.1 | Recessive, Autosomal, single-gene |

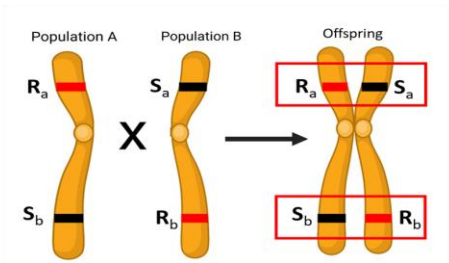
# Vip-RR Interstrain Complementation Tests

**Same locus**




Yields resistant offspring (same gene)

**Different loci**




Yields susceptible offspring (different genes)

| Insect strain cross | No. tested | Survival at Vip3Aa 10.0 ug/cm2 | Genetic Basis |
|---|---|---|---|
| F1: CBW-MS-R2-RR X CBW-TX-LT#70-RR | 256 | 0 | Different |
| F1: CBW-MS-R15-RR X CBW-TX-LT#70-RR | 256 | 0 | Different |
| F1: CBW-LA-AC4-RR X CBW-TX-LT#70-RR | 256 | 0 | Different |
| F1: CBW-LA-AC4-RR X [illegible] | 256 | 0 | Different |
| F1: CBW-MS-R2-RR X CBW-MS-R15-RR | 256 | 255 | Similar |
| F1: CBW-LA-M1-RR X [illegible] | 256 | 256 | Similar |

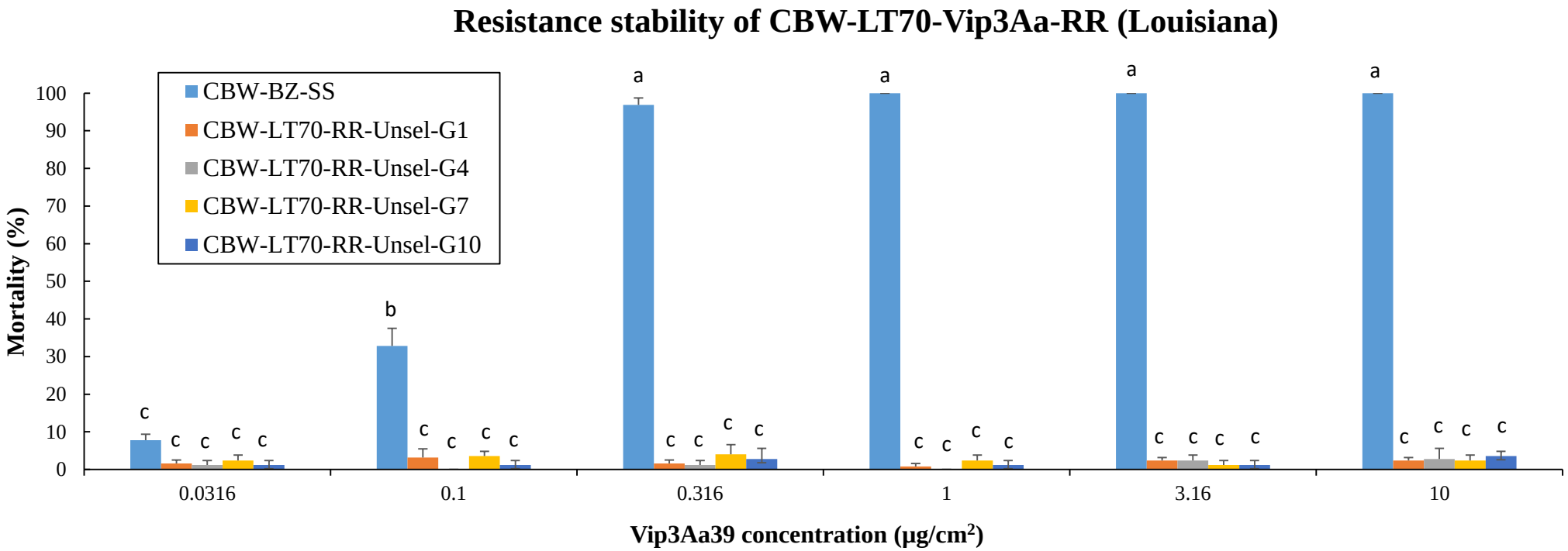
*Among these 5 strains there appears to be 3 different major gene loci conveying resistance*

*The MS strains are similar to each other* CBW-MS-R2-RR CBW-MS-R15-RR
*The TX strain is similar to one of the LA strains* CBW-TX-LT#70-RR CBW-LA-M1-RR
*One LA strain is unique* CBW-LA-AC4-RR

# Stability of Vip3Aa Resistance

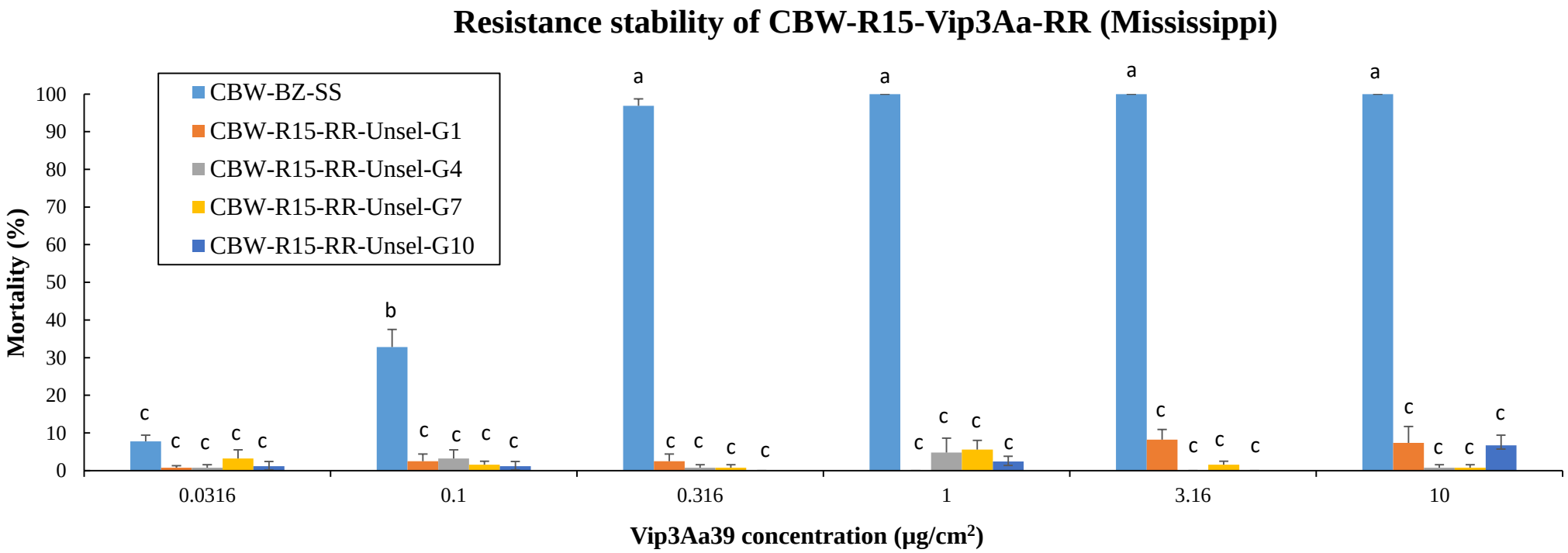
- Strains evaluated
  - CBW-TX-LT70-RR (Texas)
  - CBW-MS-R15-RR (Mississippi)
- Bt resistance selection pressure
  - No selections
- Measured mortality to Vip3Aa39 over 10 generations

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **strain** | 4 | 90 | 469.03 | <.0001 |
| **concentration** | 5 | 90 | 23.83 | <.0001 |
| **strain*concentration** | 20 | 90 | 22.06 | <.0001 |




Resistance to Vip3Aa was highly stable under no selection pressure

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **strain** | 4 | 90 | 446.97 | <.0001 |
| **concentration** | 5 | 90 | 24.88 | <.0001 |
| **strain*concentration** | 20 | 90 | 22.07 | <.0001 |




Resistance to Vip3Aa was highly stable under no selection pressure

# Triple Resistant Genotype "Super Worm" Response to Bt Proteins in Diet-overlay Bioassays

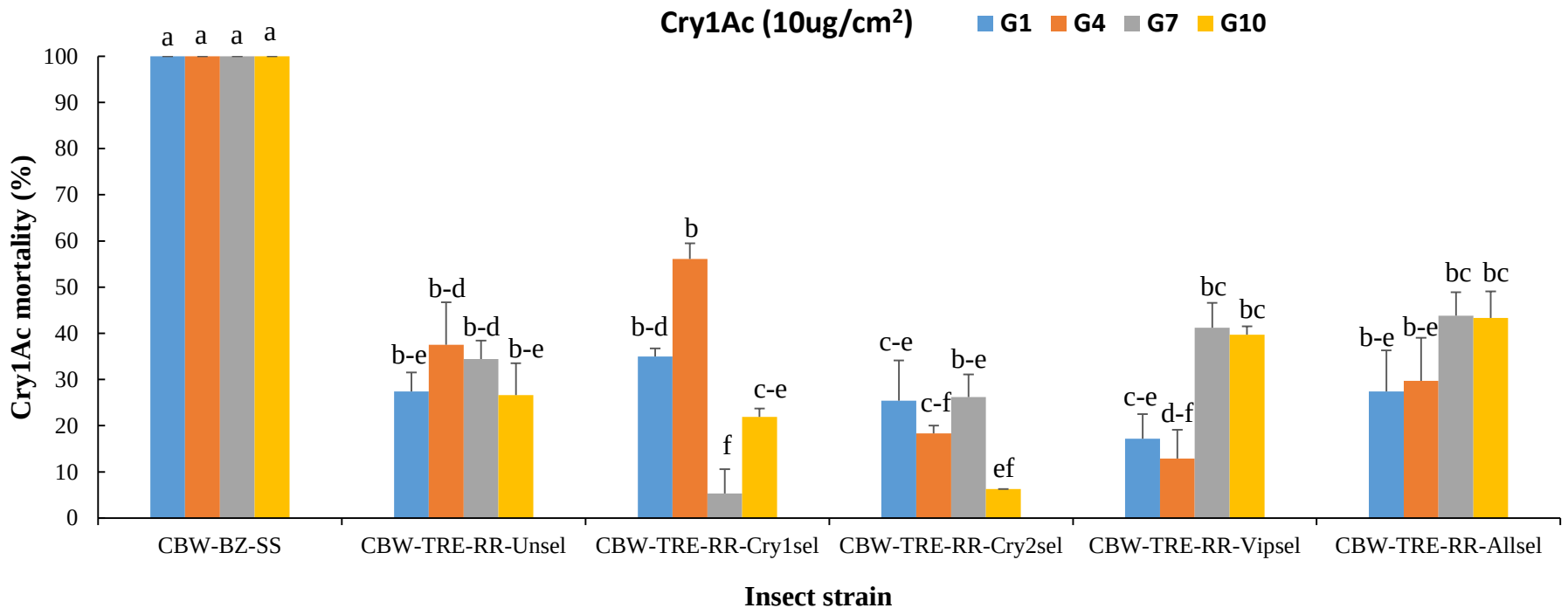
CBW-G13-Cry-RR × CBW-TX-LT#70-Vip3A-RR = Triple-RR "Super Worm"

| Bt protein | Insect strain | N[a] | $LC_{50}$ (95% CL) (µg/cm$^2$)[b] | Slope ± SE | $X^2$ | df | Resistance ratio[c] |
|---|---|---|---|---|---|---|---|
| Vip3Aa39 | CBW-BZ-SS | 512 | 0.33 (0.16, 0.78) | 1.80 ± 0.42 | 77.2 | 26 | - |
| | CBW-Triple-RR | 512 | > 10.0 | / | / | / | > 30.3 |
| Cry1Ac | CBW-BZ-SS | 512 | 0.09 (0.07, 0.12) | 1.19 ± 0.10 | 38.2 | 26 | - |
| | CBW-Triple-RR | 512 | 23.83 (10.70, 108.76) | 0.76 ± 0.14 | 19.6 | 18 | 264.8 |
| Cry2Ab2 | CBW-BZ-SS | 512 | 0.11 (0.08, 0.16) | 1.33 ± 0.13 | 36.7 | 26 | - |
| | CBW-Triple-RR | 512 | 54.48 (20.90, 419.79) | 0.73 ± 0.15 | 36.7 | 22 | 495.3 |

# Stability of Cry and Vip3Aa Resistance in a Triple Resistant Strain

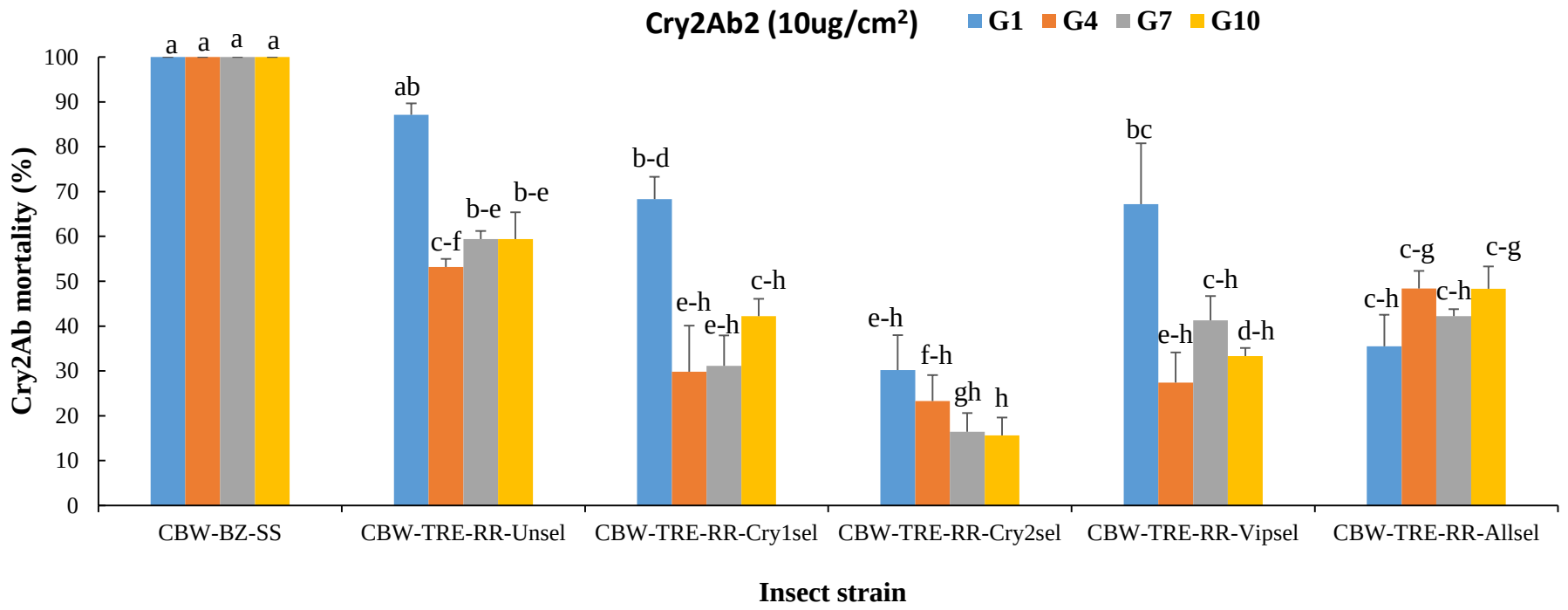
- Strain evaluated
  - CBW-TRE-RR (Triple Resistant)
- Bt resistance selection pressure
  - No selections
  - Selected every generation with Cry1Ac at 6.0 µg/cm$^2$
  - Selected every generation with Cry2Ab2 at 6.0 µg/cm$^2$
  - Selected every generation with Vip3Aa39 at 6.0 µg/cm$^2$
  - Selected every generation with Cry1Ac + Cry2Ab2 + Vip3Aa39, each at 6.0 µg/cm$^2$
- Measured mortality to Cry1Ac, Cry2Ab2, and Vip3Aa39 over 10 generations
  - Discriminating dose of each protein at 10 µg/cm$^2$
  - Full range bioassays

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **strain** | 5 | 72 | 175.93 | <.0001 |
| **Generation** | 3 | 72 | 0.33 | 0.8065 |
| **Strain*Generation** | 15 | 72 | 7.53 | <.0001 |



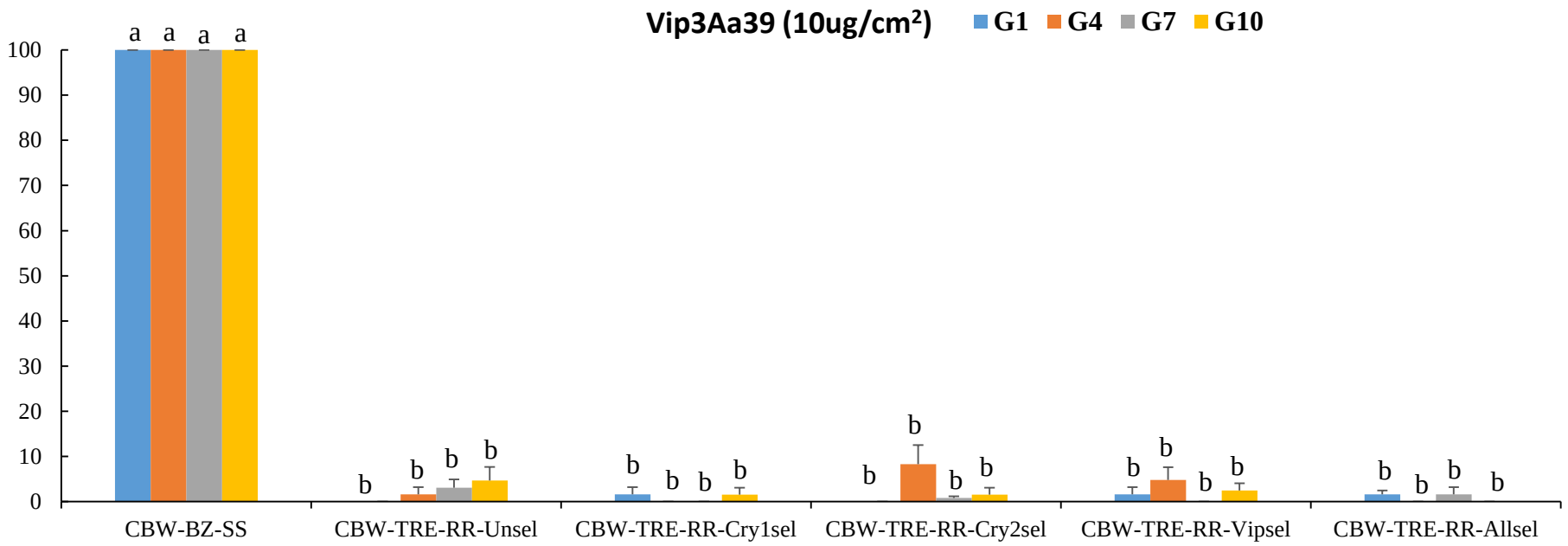

Resistance to Cry1Ac was highly stable, and did not differ from the unselected regardless of Bt protein

| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **strain** | 5 | 72 | 118.05 | <.0001 |
| **Generation** | 3 | 72 | 9.12 | <.0001 |
| **Strain*Generation** | 15 | 72 | 3.37 | 0.0003 |




All strains not selected with Cry2Ab noted a decline in susceptibility after 1 generation.

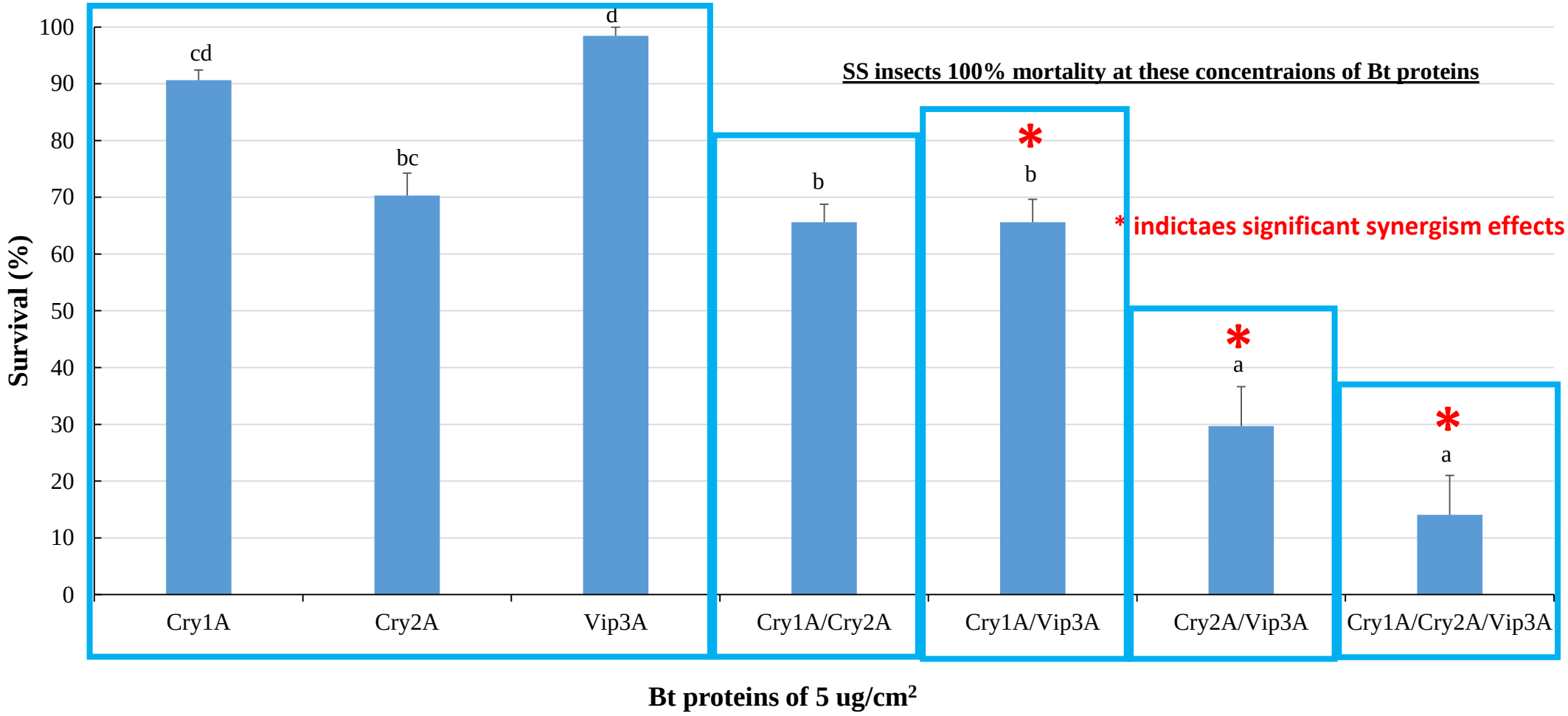
| Type III Tests of Fixed Effects | | | | |
|---|---|---|---|---|
| **Effect** | **Num DF** | **Den DF** | **F Value** | **Pr > F** |
| **strain** | 5 | 69 | 575.54 | <.0001 |
| **Generation** | 3 | 69 | 1.19 | 0.3196 |
| **Strain*Generation** | 15 | 69 | 1.78 | 0.0554 |




Resistance to Vip3Aa was highly stable, and did not differ from the unselected regardless of Bt protein

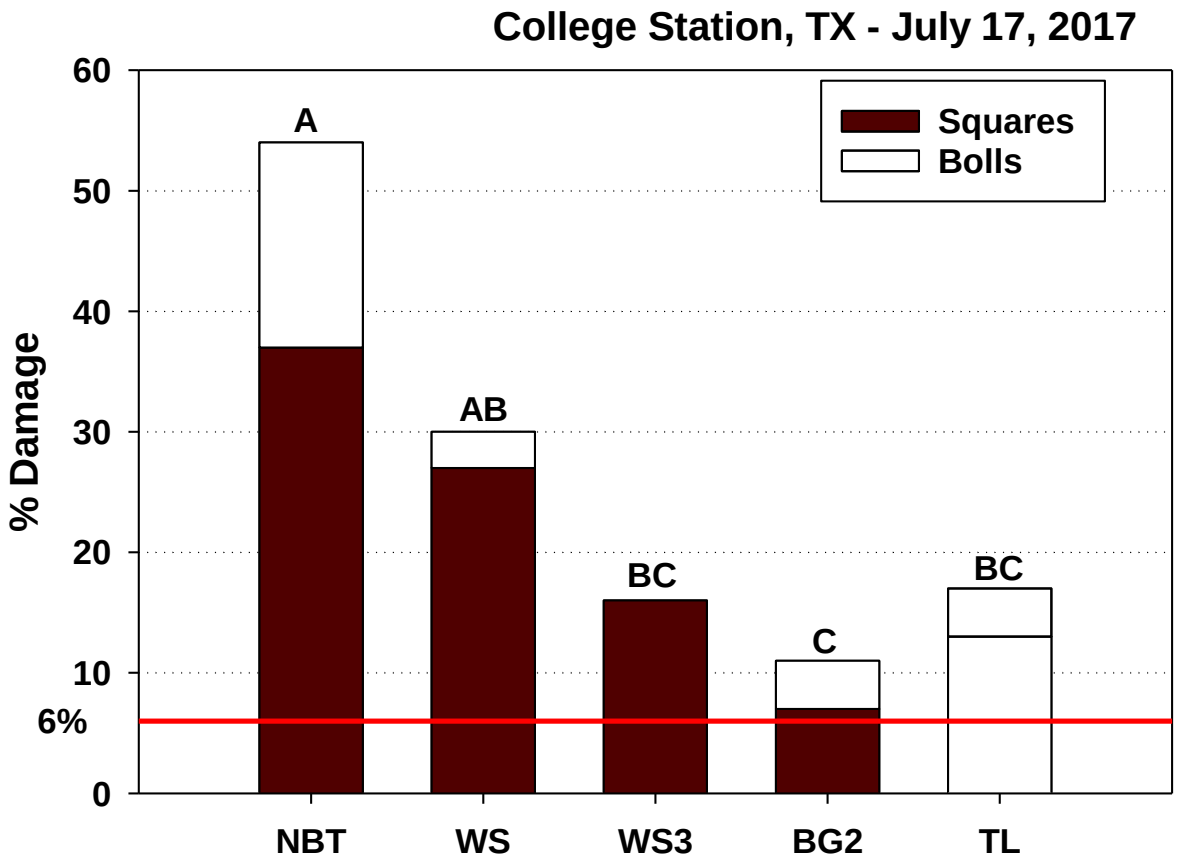
# BENEFIT OF PYRAMIDED Bt PROTEIN VS. TRIPLE GENE RESISTANT *H. zea*

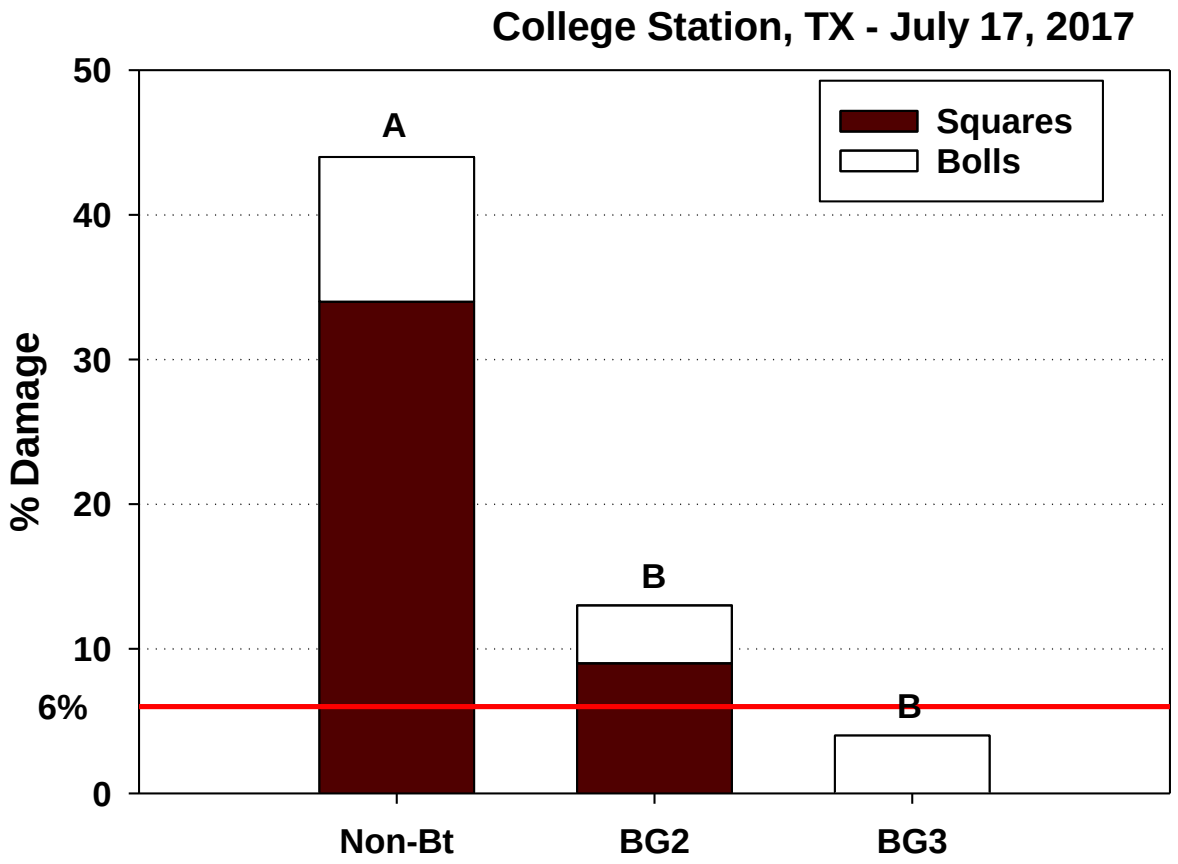
## Survival of CBW-TRE-RR-All at 5 ug/cm$^2$ of Bt proteins



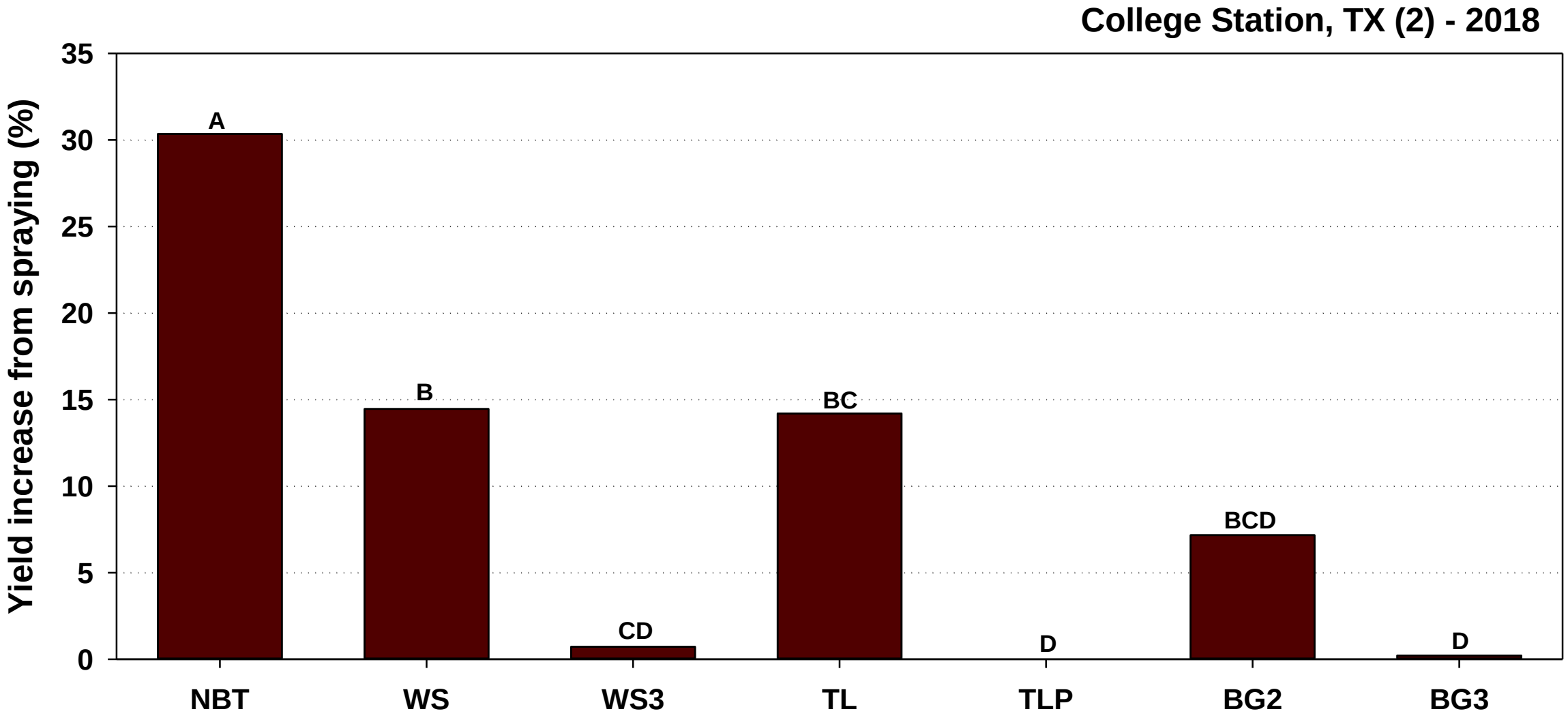

| Insect strain | Protein (5ug/cm2) | N | Observed survival (%) | No. O | Expected survival (%) | No. E | $\chi^2$ | *P*-value |
|---|---|---|---|---|---|---|---|---|
| CBW-TRE-RR-All | Cry1A/Cry2A | 64 | 65.62 | 41.9968 | 63.71 | 40.7744 | 0.100984 | 0.751 |
| CBW-TRE-RR-All | Cry1A/Vip3A | 64 | 65.62 | 41.9968 | 89.21 | 57.0944 | 36.99993 | < 0.001 |
| CBW-TRE-RR-All | Cry2A/Vip3A | 64 | 29.69 | 19.0016 | 69.21 | 44.2944 | 46.90675 | < 0.001 |
| CBW-TRE-RR-All | Cry1A/Cry2A/Vip3A | 64 | 14.06 | 8.9984 | 62.72 | 40.1408 | 64.81003 | < 0.001 |

# Bollworm Injury to Bt Cotton - High Bollworm Pressure

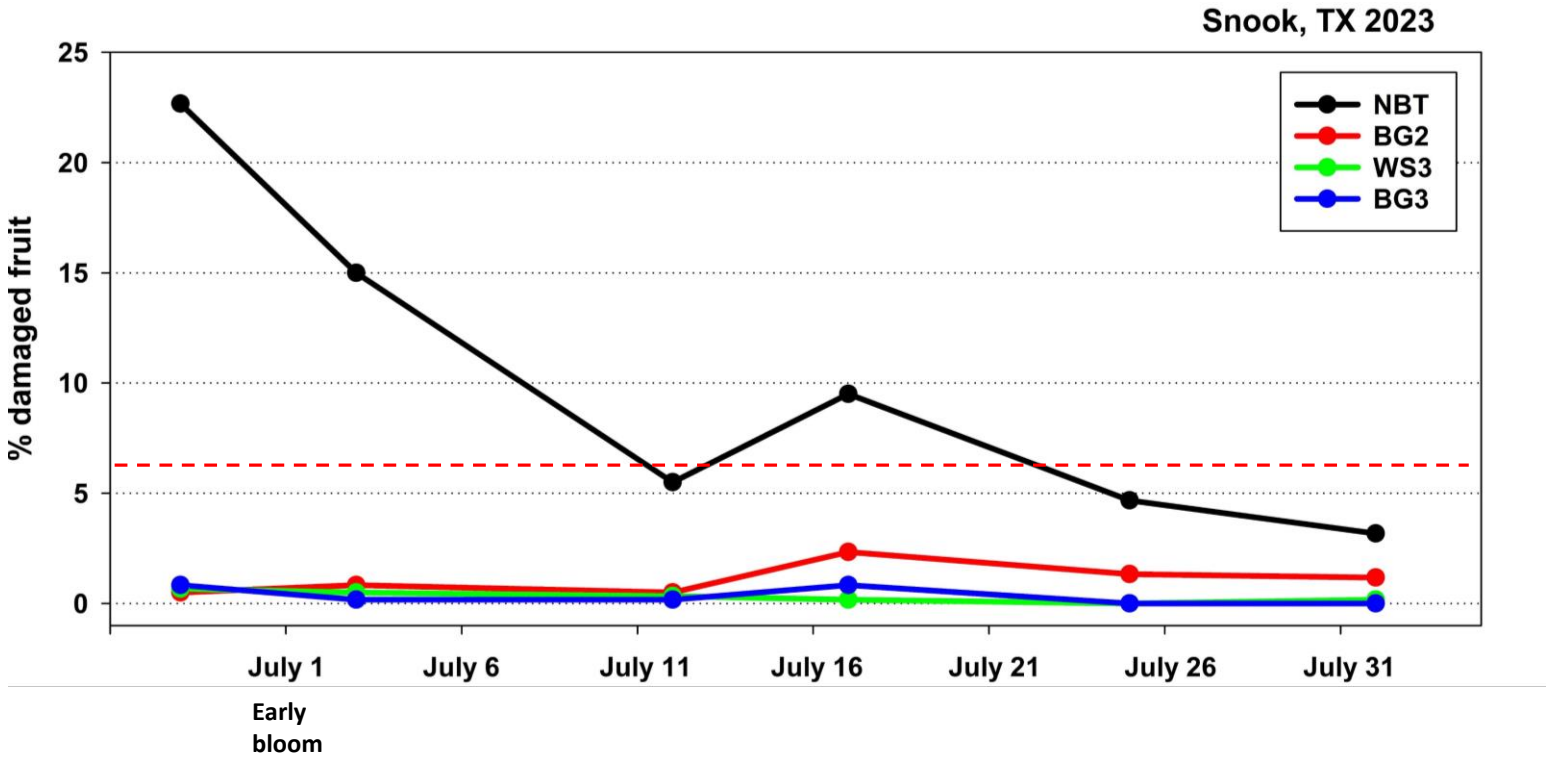


# Benefit from Spraying



# Bt Cotton Trait Performance Texas




| Percent Reduction in Fruit Damage Relative to Non-Bt | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Technology** | **June 28** | **July 3** | **July 12** | **July 17** | **July 25** | **Aug 1** | **Mean** |
| BG2 | 97.79 | 94.47 | 90.91 | 75.47 | 71.52 | 63.09 | 82.21 |
| WS3 | 97.04 | 96.67 | 94.00 | 98.21 | 100.00 | 94.64 | 96.76 |
| BG3 | 96.34 | 98.87 | 96.91 | 91.26 | 100.00 | 100.00 | 97.23 |

# 2023 Vip3Aa Cotton Unexpected Injury Events

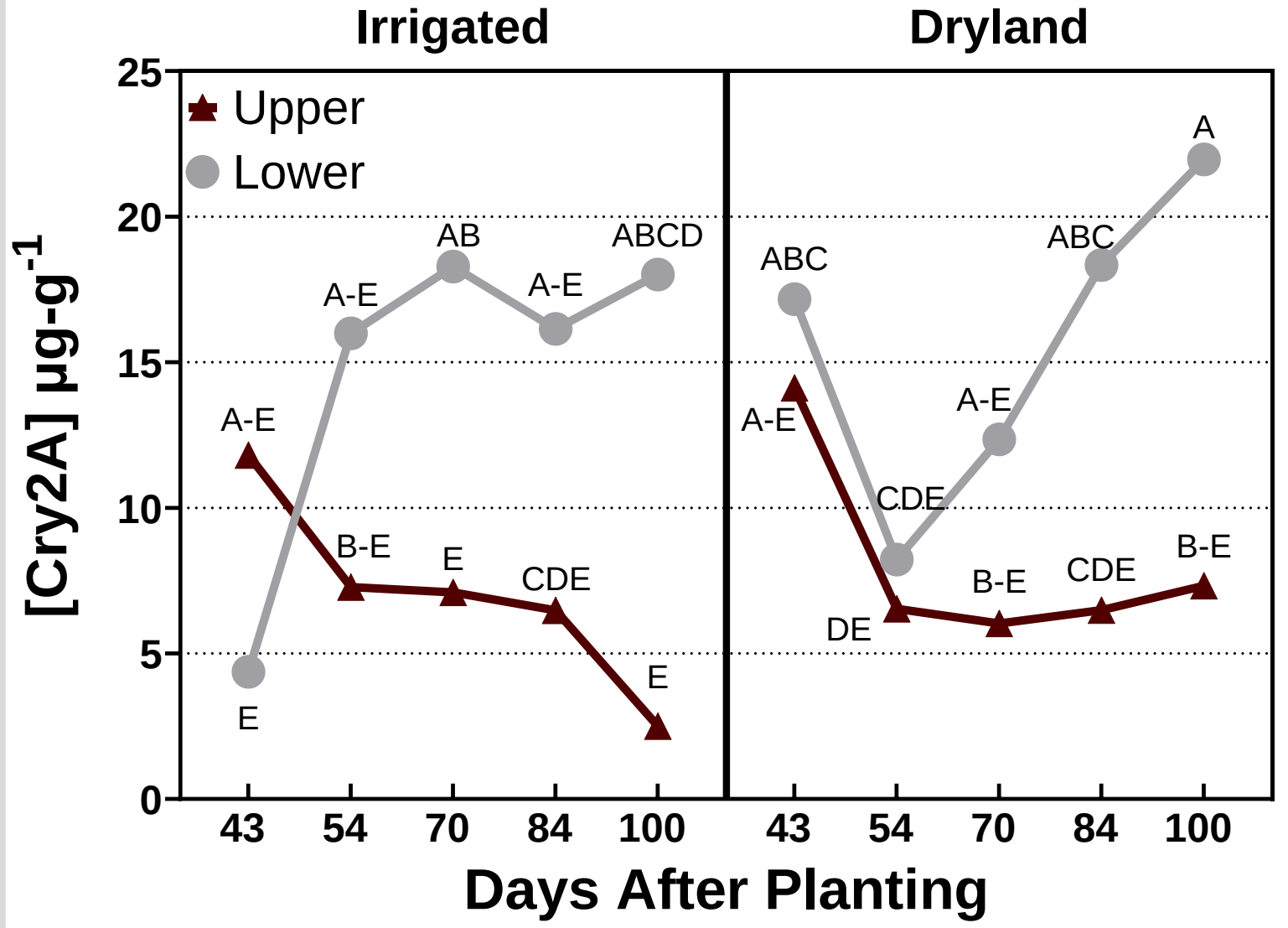
| Location | Technology | % damaged fruit | Resistance Ratio | | |
|---|---|---|---|---|---|
| | | | Cry1Ac | Cry2Ab2 | Vip3Aa39 |
| Starkville, MS | TwinLink Plus | 17% | 10298 | 1215 | 0.30 (7.50) |
| Wallis, TX | WideStrike 3 | 6% (25%) | 599.2 | 60.8 | 0.37 (9.25) |

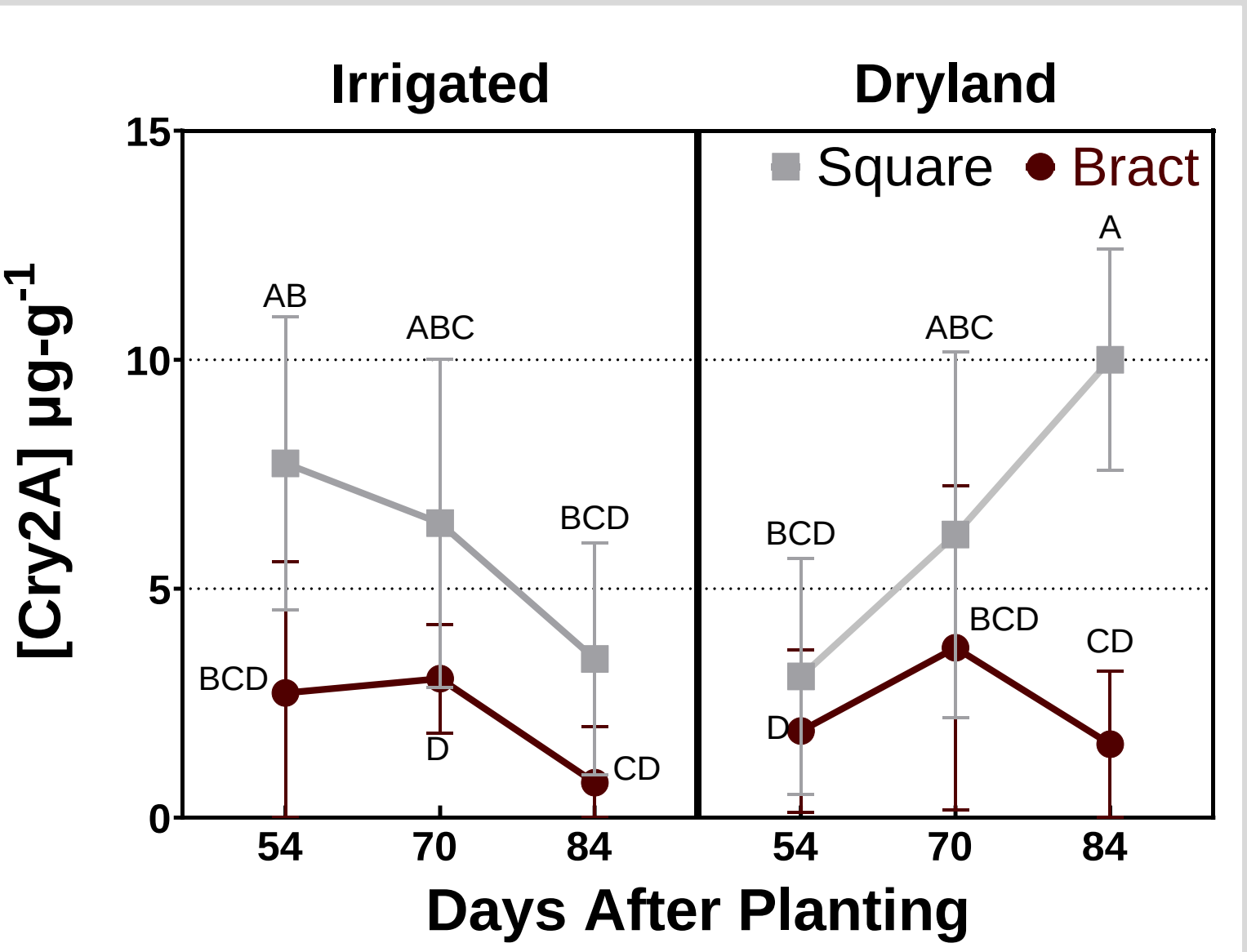
- Vip3Aa failures in 2023 occurred in cotton that was cut out
- Damage was almost exclusively to the bolls
- Vip3Aa resistance was slightly elevated but not high enough to warrant concern
- Unexpected injury was most likely associated with low Bt expression

# CRY2A CONCENTRATIONS

# CRY2A LEAF CONCENTRATIONS

- Significant three-way interactions
- Upper leaves decline as the season progressed
- 43 DAP irrigated tissues begin to separate
- 54 DAP dryland tissues begin to separate
- Perhaps Cry2A concentrations accumulate in older tissue

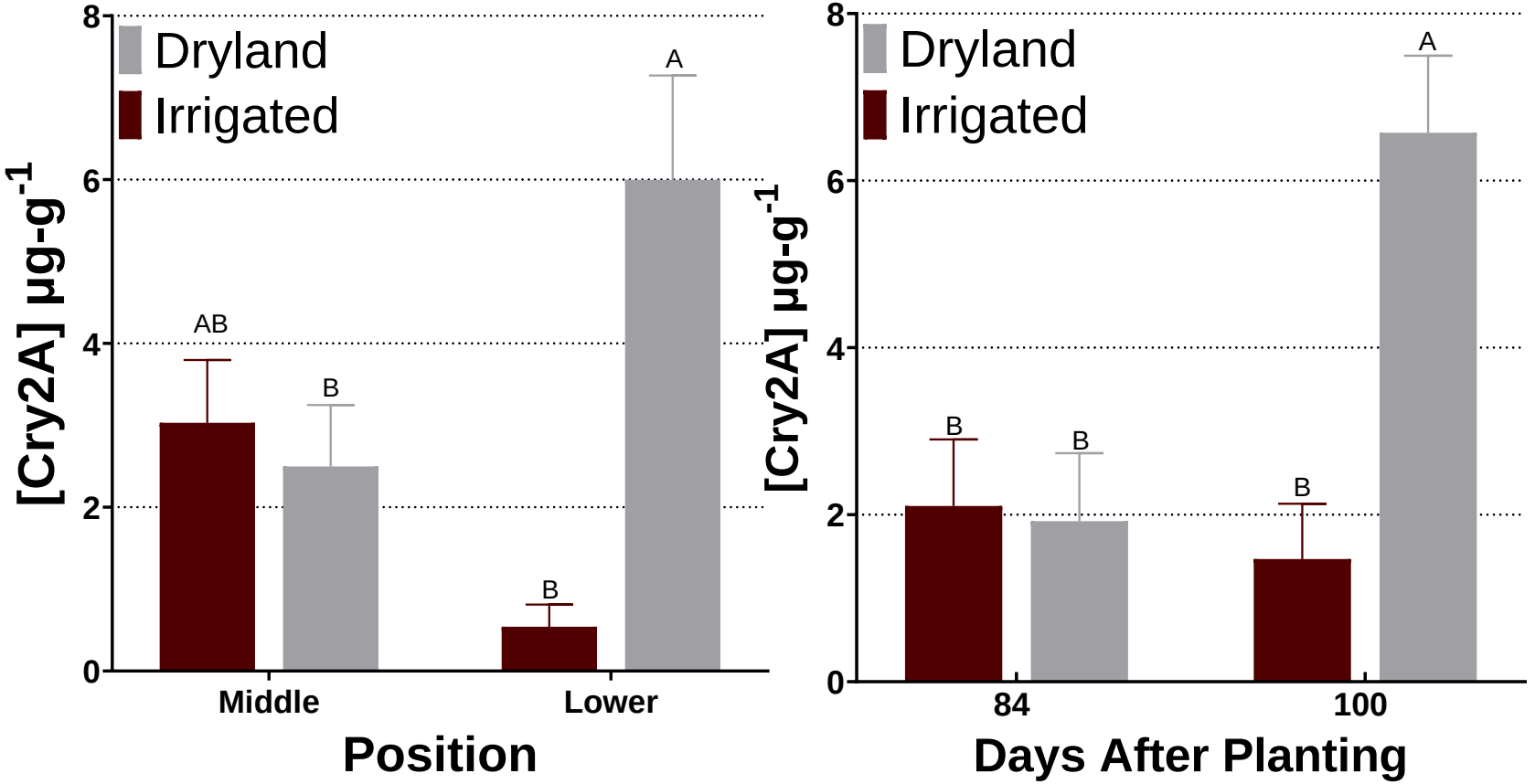





# CRY2A SQUARE CONCENTRATIONS

- No differences among different zones
- Higher Cry2A concentrations in dryland squares late in the season
- In general, square buds higher concentrations than bracts
- Overall lower concentrations than leaves

# CRY2A BOLL CONCENTRATIONS





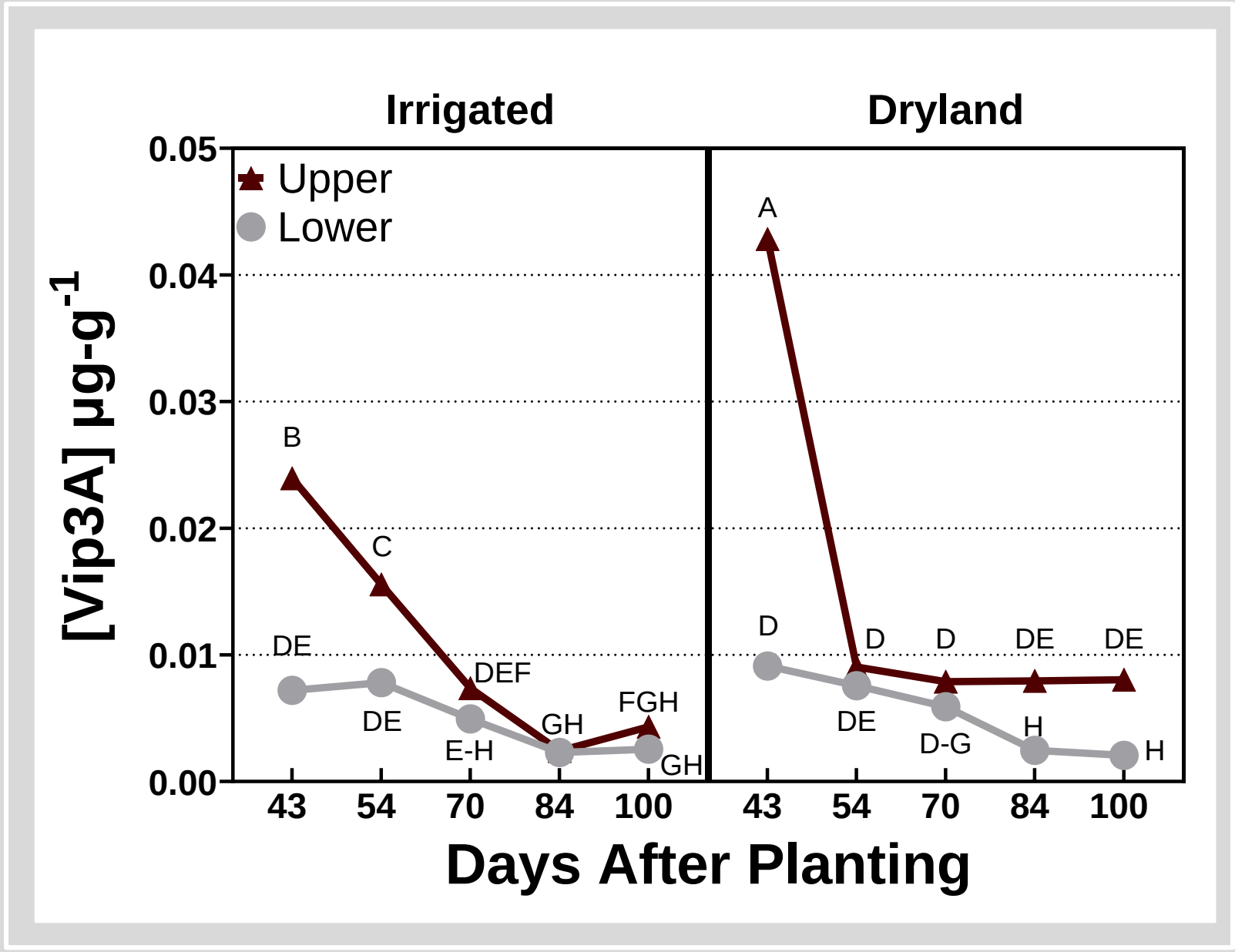


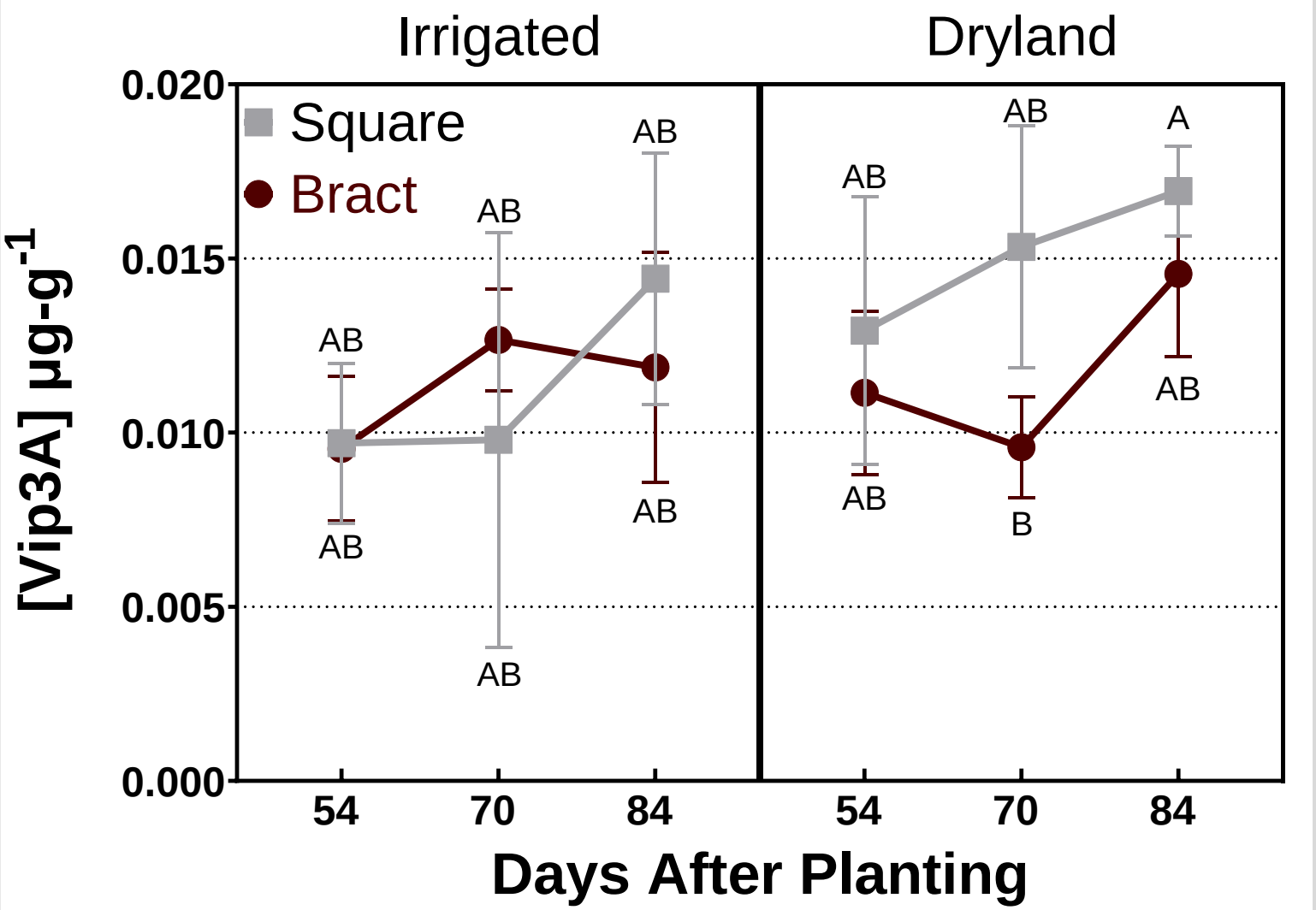
- Similar trends as leaves
- Mature lower bolls obtain higher concentrations
- Cry2A concentrations highest in mature dryland bolls
- Cry2A Concentrations in bolls significantly lower than leaves

# VIP3A CONCENTRATIONS

# VIP3A LEAF CONCENTRATIONS

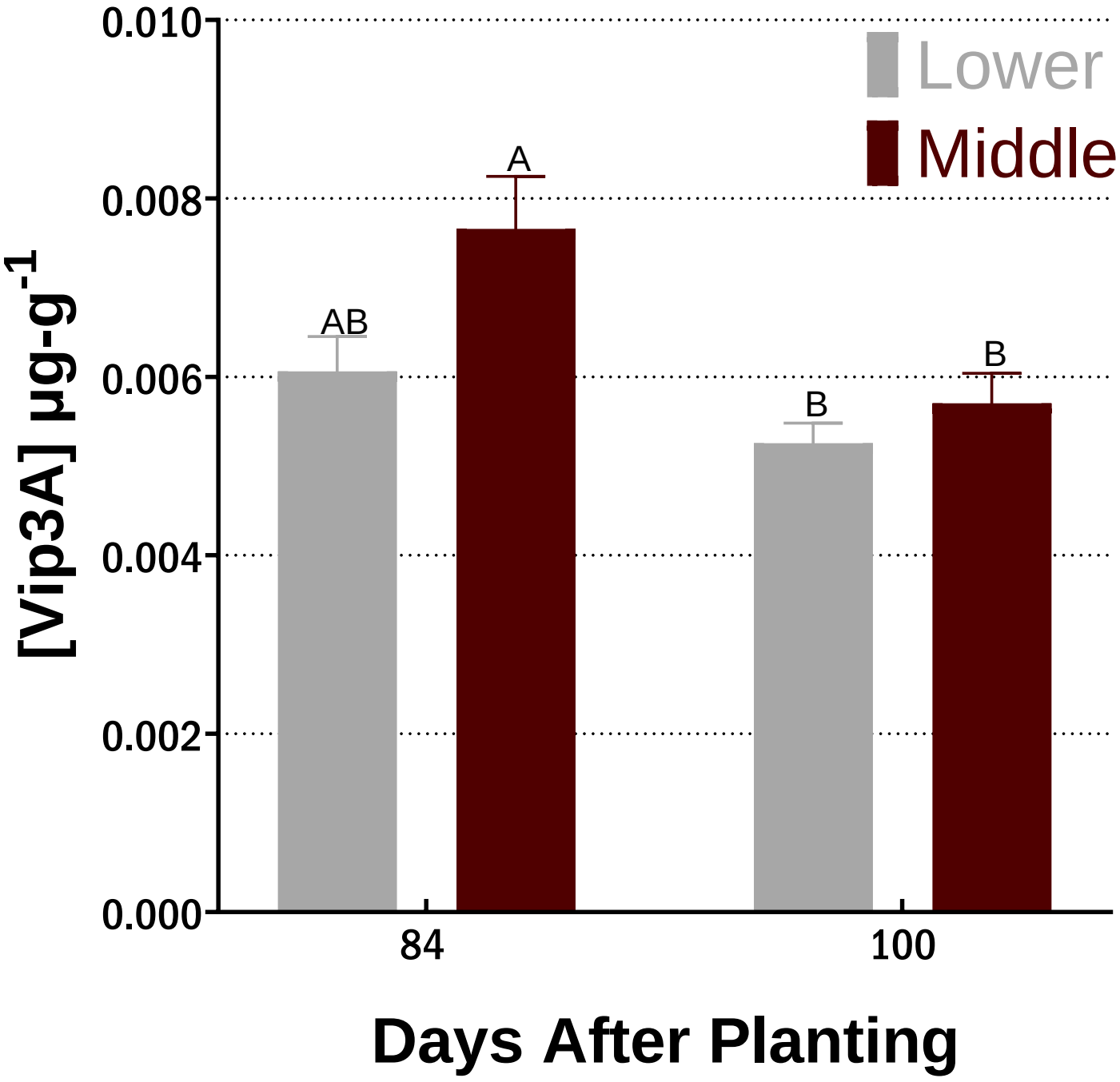
- Overall concentrations lower than Cry2A
- Significant three-way interaction
- Upper tissues statistically highest early season
- Vip3A Concentrations declined through season
- In contrast to Cry2A, Vip3A declines in all tissues through the season






# VIP3A SQUARE CONCENTRATIONS

- Vip3A concentrations similar between leaves and squares
- Essentially no differences between squares and bracts
- No significant differences between water regimes




## VIP3A BOLL CONCENTRATIONS

- Similar trends emerge
- Vip3A concentrations lower in older bolls
- Differences not discovered between dryland or irrigated
- Overall Vip3A concentrations lower in bolls compared to leaves and squares

# Thank you & Questions?

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