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FIELD PERFORMANCE OF VipCot™ IN ELITE GERMPLASM

Victor J. Mascarenhas
Syngenta Crop Protection
Leland, MS

Abstract

In 2003, VipCot, backcrossed into elite germplasm, was evaluated across the cotton belt to determine its activity against various cotton insect pests. Vip provided efficacious control of the key lepidopteran cotton pests, the tobacco budworm (*Heliothis virescens* Fabricius) and the cotton bollworm (*Helicoverpa zea* Boddie), resulting in significant yield increases compared to non-VipCot lines.

Introduction

Vip, a novel insecticidal protein derived from *Bacillus thuringiensis* (Berliner), has been recently discovered and is highly insecticidal to numerous economically important pests (Estruch *et al*. 1996, Mascarenhas *et al*. 2003). Although Vip is derived from *Bacillus thuringiensis* (Berliner), several factors separates it from the various delta-endotoxins reported in the literature, such as the CryIAc found in Bt cotton. Lee *et al*. (2003) discusses the various ways in which Vip3A differ from Cry1Ab delta-endotoxin. Vip is a protein that is secreted during the vegetative stages of bacterial development (Estruch *et al*. 1996, Yu *et al*. 1997) thus it is classified as an exotoxin. In contrast, CryIA proteins are only found during the sporulation phase and are classified as endotoxins. Furthermore, delta-endotoxins are in a crystallin phase, which requires solubilization before it can be activated by midgut proteases. Vip is already in a soluble state, thus is more readily available to bind to midgut receptors of susceptible insects. In addition, Estruch *et al*. (1996) reported no sequence or structural homology between Vip and delta-endotoxins.

Reported here are results of several field studies designed to assess the efficacy and spectrum of control obtained from cotton plants that have been genetically modified to express the Vip gene.

Methods and Materials

Performance of VipCot was evaluated across various locations throughout the cotton belt in 2003. A total of six internal and two university cooperator trials were conducted to investigate the efficacy of the VipCot trait that has been backcrossed into elite cotton germplasms. The trials were conducted in Texas, Louisiana, Mississippi, Arkansas, Georgia, South Carolina and North Carolina. Data presented in this manuscript include trials from the following cooperators: Roger Leonard - LSU and J. R. Bradley – NCSU. In most locations, plots were eight rows by 30 ft in length and replicated four times in a RCB design. VipCot backcrosses (DPLX-Vip and DPLY-Vip) and its parent isoline without VipCot (DPLX and DPLY) were evaluated in side-by-side comparisons with and without additional insecticide applications made for Lepidopteran control. Reported here are the results observed when no additional lepidoptericide applications were made. All other non-lepidopteran insect pests were managed on an "as needed" basis with narrow spectrum insecticides. Lepidopteran insect populations and their damage to cotton structures were monitored throughout the growing season. Sampling regimes varied across locations. In most cases, percent infestation and percent damage in squares, bloom tags and bolls were estimated by sampling 25 to 50 structures per plot per assessment date. Species composition (tobacco budworm versus cotton bollworm) was estimated in each location at various time intervals during the growing season. Yield was estimated by box-mapping 20 foot of row from each plot. Data are presented as cumulative numbers over the course of the season. Data were subjected to ANOVA, and means were separated according to Student-Newman-Keuls (P= 0.05).

Results

Cumulative Infestation and Damage

Although most locations reported nearly discrete bollworm populations for most of the season (Haskell-TX, Newport-AR, Leland-MS, Hartsville-SC and Jamesville, NC), other locations reported a mixture of the Heliothine complex with some locations shifting from bollworm early season to predominantly budworm during late season (Winnsboro-LA and Quitman, GA). In addition, insect pressure and duration of moth flights varied widely from one location to the next.

In all locations where sufficient insect pressure was present, VipCot significantly reduced numbers of larvae on squares compared with non-VipCot lines (Table 1). Percent square infestation in VipCot lines (DPLX-Vip and DPLY-Vip) ranged from 0 to 2.3% compared to 1 to 12.1% in non-VipCot lines. Averaged across all location and VipCot lines, VipCot reduced square infestation by 81%. In addition, VipCot significantly reduced cumulative percent damaged squares across all locations (Table 1). Percent damaged squares ranged from 0.5 to 14.9% in VipCot lines compared to 8.5 to 34.7% in non-VipCot

lines. Averaged across locations and VipCot lines, VipCot reduced square damage by 74%. VipCot also effectively protected young bolls developing under dried stuck flowers, commonly referred to as bloom tags (Table 2). The cumulative percent of bloom tags infested with larvae in VipCot lines ranged from 0 to 1.6% compared with 2.5 to 29.9% in non-VipCot (Table 2). Averaged across locations and VipCot lines, VipCot reduced bloom tag damage by 92%. In addition, VipCot significantly reduced the cumulative percent infestation on bolls compared with non-VipCot lines (Table 3). Percent boll infestation in VipCot lines ranged from 0 to 2.5% compared to 1 to 13.3% in non-VipCot lines. Averaged across locations and VipCot lines, VipCot reduced boll damage by 70%.

Yield

VipCot lines yielded significantly more lint cotton than their parent isolines (Table 4). VipCot yields ranged from 473 to 1,545 lb lint per acre compared with 0 to 1095 lb lint per acre for non-VipCot. Averaged across all locations and VipCot lines, yields for VipCot and non-VipCot were 1,017 and 609 lb, respectively. These differences represent an average increase of 408 lb of lint cotton per acre due to VipCot.

Discussion

In these field trials, VipCot significantly reduced infestation levels of the Heliothine complex and effectively reduced the level of damage to squares, bloom tags and bolls by an average of 82% compared to non-VipCot. The observed reduction in numbers of larvae and damage to fruiting body indicates a robust insecticidal protein expression throughout the plant structures that are critical to yield. The protection of these fruiting structures directly translated in significant yields across all locations. In the Quitman, GA location where high insect pressure occurred late in the season, VipCot lines produced an average of 558 lb more lint cotton than the non-Vip lines. In addition to providing excellent protection against a broad range of lepidopteran pests, VipCot represents a novel insecticidal protein that is structurally distinct and with a different mode of action from delta-endotoxin proteins currently available. These attributes enable VipCot to have a unique fit into IPM systems in cotton, as well as resistance management strategies for all Bt derived insecticidal proteins.

References

Estruch J.J., G.W. Warren, M.A. Mullins, G.J. Nye, J.A. Craig and M.G. Koziel. 1996. Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. Proceedings National Academy of Science USA. Vol. 93(11): 5389-94.

Lee, M.K., F.S. Walters, H. Hart, N Palekar and J.S. Chen. 2003. The mode of action of *Bacillus thuringiensis* vegetative insecticidal protein Vip3A differs from that of Cry 1Ab delta-endotoxin. Applied and Environmental Microbiology. Vol. 69 (8): 4648-4657.

Mascarenhas V.J., R. Boykin, F. Shotkoski. 2003. Field performance of Vip cotton against various lepidopteran cotton pests in the U.S., pp. . *In* P. Dugger and D. A. Richter [eds.], Proceedings, 1998 Beltwide Cotton Conference. National Cotton Council, Memphis, TN.

Yu C.G, M.A. Mullins, G.W. Warren, M.G. Koziel, and J.K.J. Estruch. 1997. The *Bacillus thuringiensis* vegetative insecticidal protein Vip3A lyses midgut epithelium cells of susceptible insects. Applied and Environmental Microbiology. Vol. 63(2): 532-536.

Table 1: Species composition of the Heliothine complex at each trial location during 2002.

	H. virescens	H. zea	Sampling Period	Efficacy Assessment
Internal Trials				
Waco, TX	0%	100%	Season Long	7/06/02 to 8/20/02
Winnsboro, LA	5%	95%	7/30/02	7/12/02 to 8/26/02
	45%	55%	8/14/02	
	98%	2%	8/22/02	
Leland, MS	67%	33%	7/27/02	6/30/02 to 9/05/02
	50%	50%	8/11/02	
	71%	29%	8/22/02	
	86%	14%	8/29/02	
	88%	12%	9/05/02	
Beasley, TX	0%	100%	6/03/02	6/11/02 to 8/27/02
	28%	72%	6/29/02	
	45%	55%	7/09/02	
	96%	4%	7/29/02	
Brooks Co., GA	0%	100%	7/04/02	7/01/02 to 8/15/02
	60%	40%	7/24/02	
	93%	7%	8/22/02	
Newport, AR	2%	79%	Season Long	7/31/02 to 8/26/02
Houston Co., AL	0%	100%	Season Long	7/03/02 to 9/12/02
Cooperator Trials				
Winnsboro, LA	50%	50%	7/04 - 12/02	7/10/02 to 9/12/02
	0%	100%	7/17 - 24/02	
	0%	100%	8/02 - 12/02	
	71%	29%	9/07/02	
	67%	33%	9/18/02	
Corpus Christi, TX	80%	20%	Early/mid-season	7/15/02 to 8/22/02
	100%	0%	Late season	
Jamesville, NC	0%	100%	Season Long	7/31/02 to 8/19/02

Table 2: Percent infestation based on the cumulative number of Heliothine eggs observed on cotton terminals.

	Vip	Coker
Internal Trials		
Winnsboro, LA	21.0 a	18.8 a
Newport, AR	7.6 a	4.8 a
Beasley, TX	13.9 a	13.8 a
Houston Co., AL	8.9 a	9.3 a
Cooperator Trial		
Winnsboro, LA	10.8 a	9.8 a

Means within a row followed by the same letter do not differ significantly according to Student-Newman-Keuls (P = 0.05).

Table 3: Cumulative percent Heliothine infestation and damage on terminals of Vip and Coker cotton.

	Cumulative Percent Infestation		Cumulative Percent Damage	
	Vip	Coker	Vip	Coker
Internal Trials				
Winnsboro, LA	4.3 b	11.2 a	10.9 b	18.1 a
Waco, TX	3.5 b	34.5 a	5.0 b	41.5 a
Newport, AR	3.5 b	9.5 a	28.5 b	44.5 a
Houston Co., AL	0.0 b	5.6 a	--	--
Cooperator Trials				
Winnsboro, LA	0.2 a	1.4 a	--	--
Corpus Christi, TX	0.0 b	10.0 a	10.0 b	71.5 a

Means within a row followed by the same letter do not differ significantly according to Student-Newman-Keuls (P = 0.05).

Table 4: Cumulative percent Heliothine infestation and damage on squares of Vip and Coker cotton.

	Cumulative Percent Infestation		Cumulative Percent Damage	
	Vip	Coker	Vip	Coker
Internal Trials				
Winnsboro, LA	0.7 b	4.7 a	2.9 b	14.7 a
Waco, TX	6.0 b	34.0 a	12.2 b	69.7 a
Newport, AR	0.0 b	4.0 a	5.2 b	20.3 a
Beasley, TX	0.6 b	6.9 a	2.0 b	23.4 a
Leland, MS	1.7 b	6.4 a	3.0 b	22.3 a
Houston Co., AL	--	--	0.0 b	6.2 a
Brooks, GA	--	--	4.1 b	35.4 a
Cooperator Trials				
Winnsboro, LA	1.0 b	5.1 a	1.7 b	13.5 a
Corpus Christi, TX	2.0 b	18.5 a	1.2 b	10.7 a
Jamesville, NC	1.5 b	11.0 a	4.0 b	35.6 a
Tift Co., GA	0.0 a	2.5 a	--	--

Means within a row followed by the same letter do not differ significantly according to Student-Newman-Keuls (P = 0.05).

Table 5: Cumulative percent Heliothine infestation and damage on flowers of Vip and Coker cotton.

	Cumulative Percent Infestation		Cumulative Percent Damage	
	Vip	Coker	Vip	Coker
Internal Trials				
Winnsboro, LA	2.5 b	7.7 a	3.6 b	14.4 a
Waco, TX	20.7 b	45.0 a	9.3 b	44.0 a
Newport, AR	1.7 b	7.5 a	3.0 b	15.3 a
Beasley, TX	1.7 b	11.0 a	4.9 b	17.4 a
Leland, MS	1.7 b	6.4 a	1.6 b	16.6 a
Brooks, GA	--	--	5.3 b	36.2 a
Cooperator Trials				
Winnsboro, LA	2.8 a	10.6 a	3.1 b	15.9 a
Jamesville, NC	1.5 b	11.0 a	2.6 b	64.0 a

Means within a row followed by the same letter do not differ significantly according to Student-Newman-Keuls (P = 0.05).

Table 6: Cumulative percent Heliothine infestation on bloom tags and damage to apical portions of bolls covered by bloom tags of Vip and Coker cotton.

	Cumulative Percent Infestation		Cumulative Percent Damage	
	Vip	**Coker**	**Vip**	**Coker**
Internal Trials				
Beasley, TX	1.4 b	15.1 a	3.5 b	35.9 a
Cooperator Trials				
Winnsboro, LA	2.2 b	10.9 a	1.6 b	20.0 a

Means within a row followed by the same letter do not differ significantly according to Student-Newman-Keuls (P = 0.05).

Table 7: Cumulative percent Heliothine infestation and damage on bolls of Vip and Coker cotton.

	Cumulative Percent Infestation		Cumulative Percent Damage	
	Vip	**Coker**	**Vip**	**Coker**
Internal Trials				
Winnsboro, LA	2.5 b	6.5 a	4.8	21.8 a
Waco, TX	2.0 b	41.5 a	5.7	52.7 a
Newport, AR	0.4 b	3.1 a	2.8	18.1 a
Beasley, TX	0.8 b	9.6 a	5.5	33.9 a
Leland, MS	--	--	4.4	13.5 a
Houston Co., AL	--	--	0.6	3.2 a
Brooks, GA	--	--	6.5	49.2 a
Cooperator Trials				
Winnsboro, LA	0.7 b	5.3 a	1.8	16.1 a
Jamesville, NC	3.0 b	22.0 a	8.2	66.5 a

Means within a row followed by the same letter do not differ significantly according to Student-Newman-Keuls (P = 0.05).

Table 8: Levels of beet armyworm larvae infesting Vip and Coker cotton.

	Locations and Sampling Unit				
	Corpus Christi, TX No. larvae/10 leaves	**Newport, AR No. larvae/12 row ft**	**Leland, MS No. larvae/12 row ft**	**Brooks, GA N0. larvae/60 fruit**	**Beasley, TX No. hits/16 rows**
Vip	0.0 b	0.6 b	1.0 b	1.2 b	1.4 b
Coker	1.5 a	5.6 a	10.5 a	23.7 a	15.1 a

Means within a row followed by the same letter do not differ significantly according to Student-Newman-Keuls (P = 0.05).

Table 9: Levels of soybean looper larvae infesting Vip and Coker cotton.

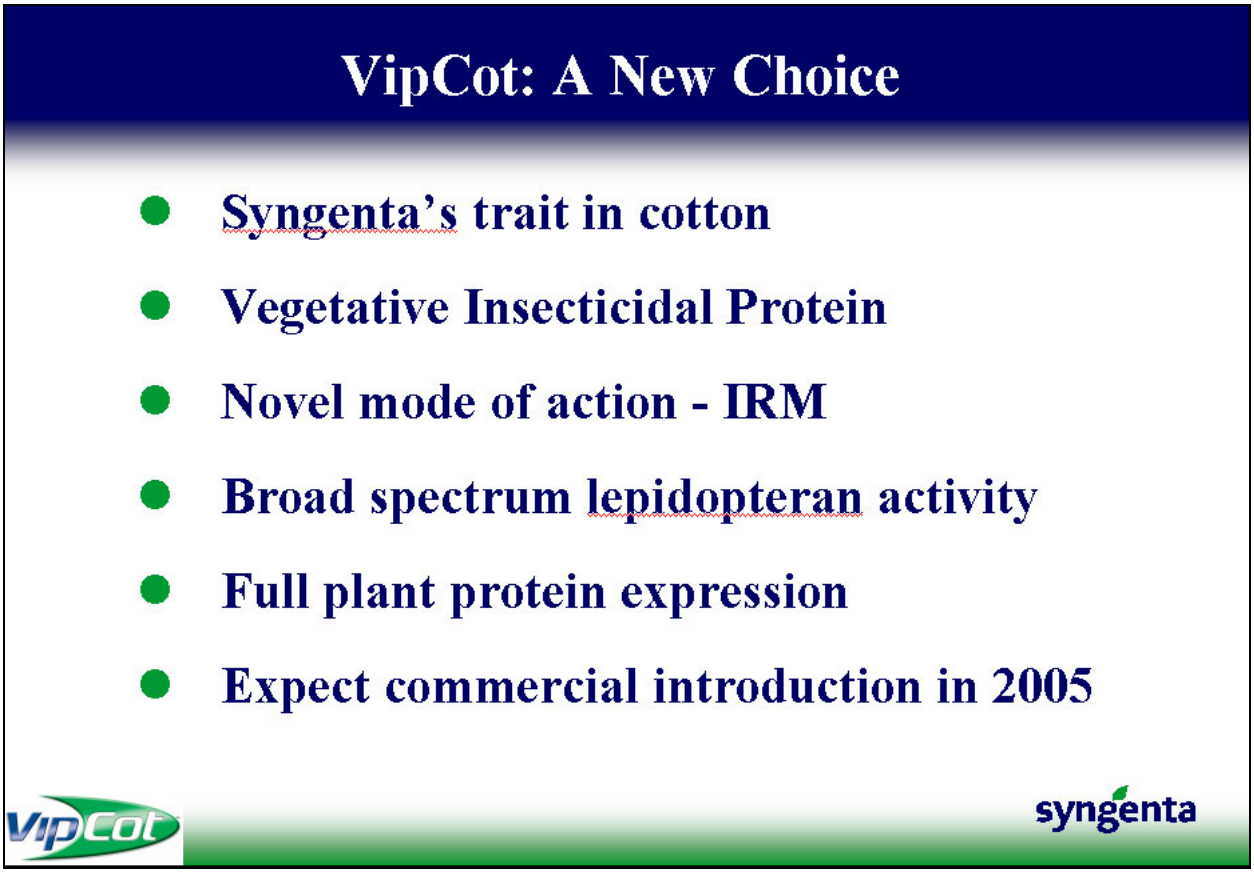
	Locations and Sampling Unit		
	Winnsboro, LA No. larvae/2 sweeps	**Newport, AR N0. larvae/row ft**	**Leland, MS No. larvae/row ft**
Vip	2.8 b	0.2 a	0.2 b
Coker	11.8 a	0.5 a	6.9 a

Means within a row followed by the same letter do not differ significantly according to Student-Newman-Keuls (P = 0.05).

Table 10: Seed cotton yield expressed as lb seed cotton/acre.

	Vip	Coker
Internal Trials		
Winnsboro, LA	1378 a	975 b
Waco, TX	2210 a	459 b
Newport, AR	2138 a	1605 b
Beasley, TX	1912 a	635 b
Leland, MS	2629 a	1213 b
Houston Co., AL	1247 a	862 b
Brooks, GA	1646 a	1423 b
Cooperator Trials		
Winnsboro, LA	2237 a	1306 b
Jamesville, NC	2526 a	742 b
Tift Co., GA	1485 a	1425 b

Means within a row followed by the same letter do not differ significantly according to Student-Newman-Keuls (P = 0.05).



Vip: Spectrum of Activity

SUSCEPTIBLE	NON-SUSCEPTIBLE
• Black cutworm	• European corn borer
• Corn earworm	• Diamondback moth
• Tobacco budworm	• Monarch butterfly
• Pink bollworm	• Saltmarsh
• Fall armyworm	• Western corn rootworm
• Beet armyworm	• Northern corn rootworm
• Yellow striped armyworm	• Southern corn rootworm
• Southwestern corn borer	• Colorado potato beetle
• Sugarcane borer	• Yellow meal worm
• Mediterranean corn borer	• Northern house mosquito
• Cabbage looper	• House fly
• Soybean looper	• Fruit fly
• Velvet bean caterpillar	• Collembola
• Cotton leaf perforator	

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VipCot: A New Choice

- Field tested since 2000 in Coker background
- Evaluated in many cotton markets worldwide to include China, India, Australia and USA
- 2003 evaluated performance in elite germplasm in cooperation with seed company
- Report on portion of the efficacy results for 2003

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Materials & Methods

- Total of 8 trials in collaborative effort with D&PL:
  - Internal trials – TX, AR, MS (2), GA & SC
  - University – LA (R. Leonard) & NC (J.R. Bradley)
- Two backcross material
- Larval infestation and damage to terminals, squares, flowers, bloom tags and bolls were monitored throughout the season
- Assessment varied by location (25-50 structures)
- Yield was estimated from box mapping samples
- Data presented as cumulative % infestation and damage across all assessments.

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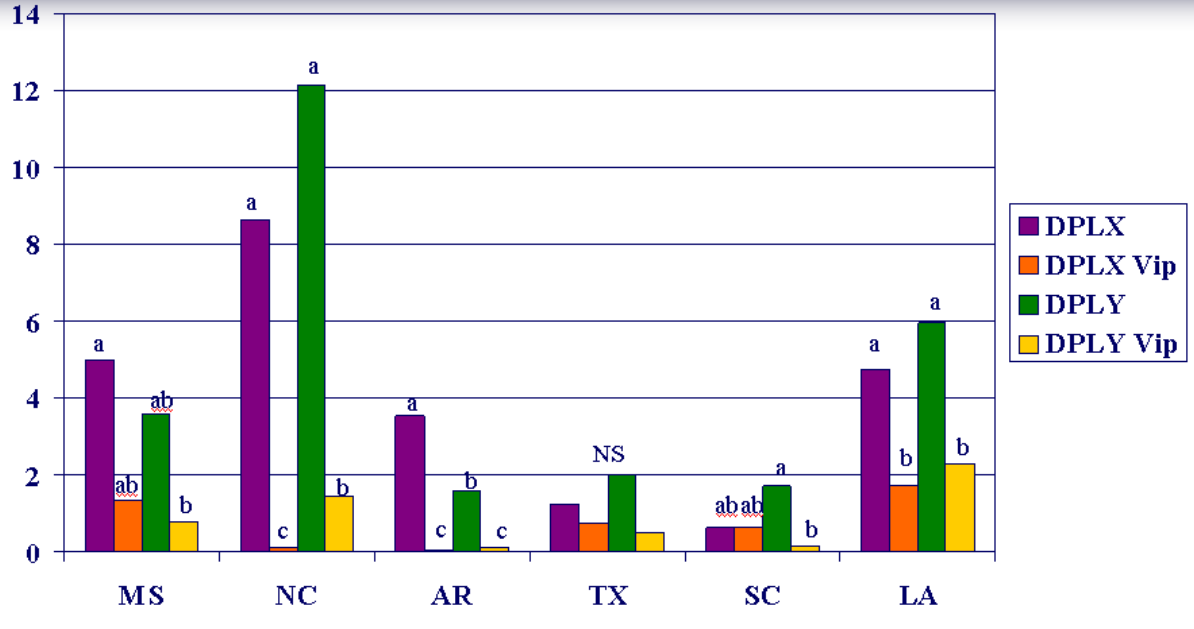
Cumulative Percent Infested Squares



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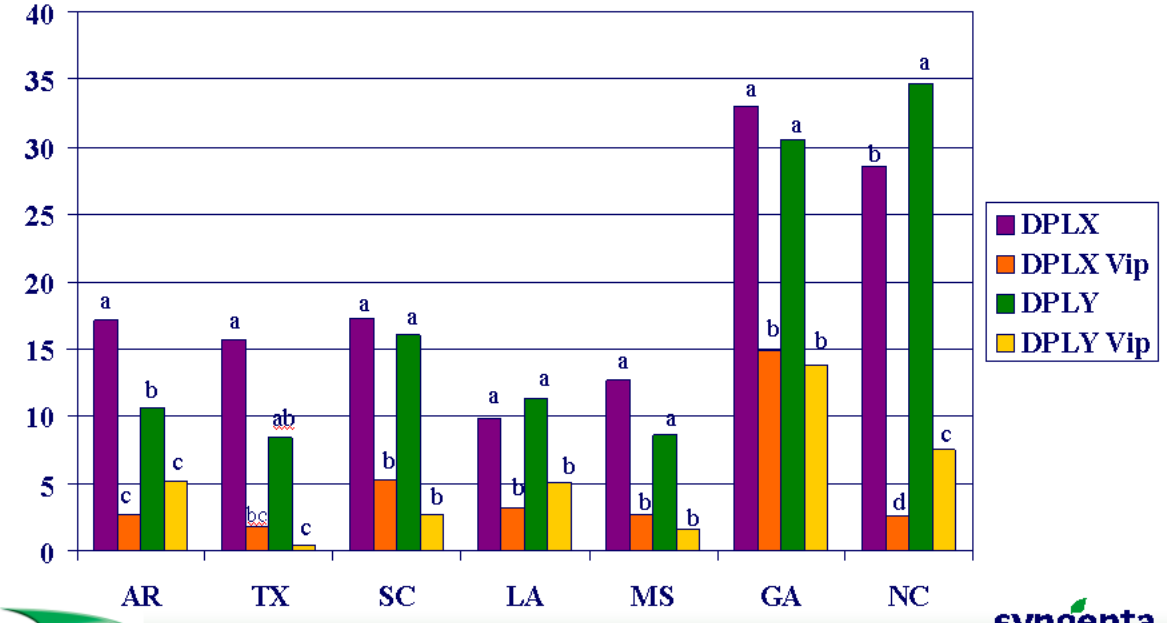
Cumulative Percent Damaged Squares



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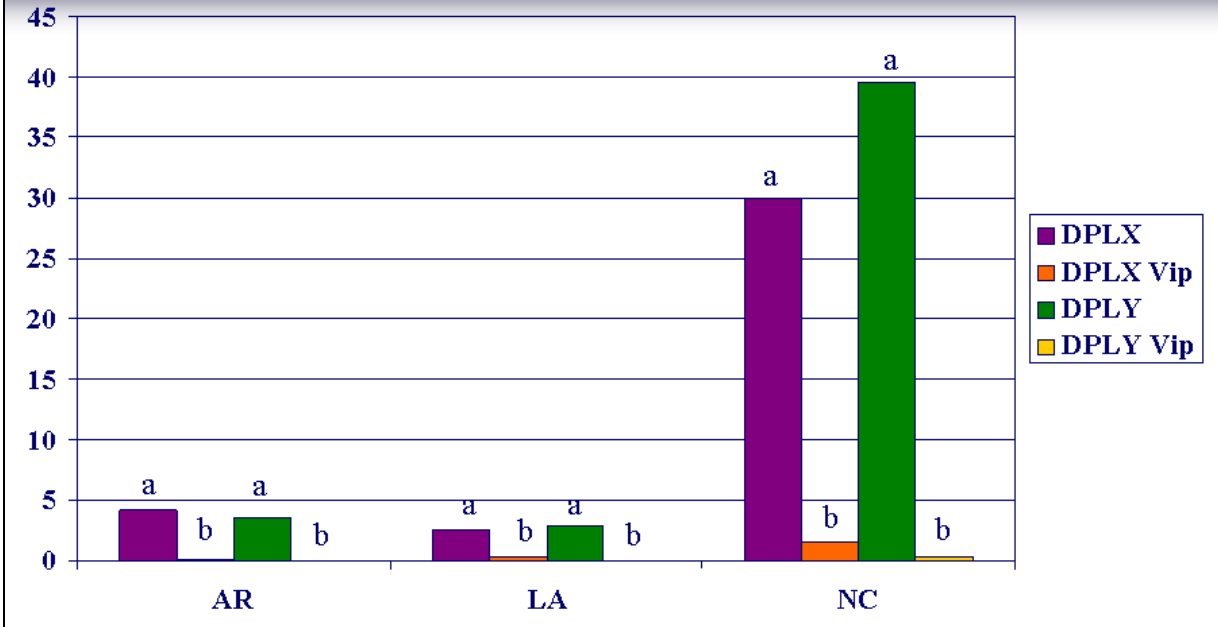
Cumulative Percent Infested Bloom Tag



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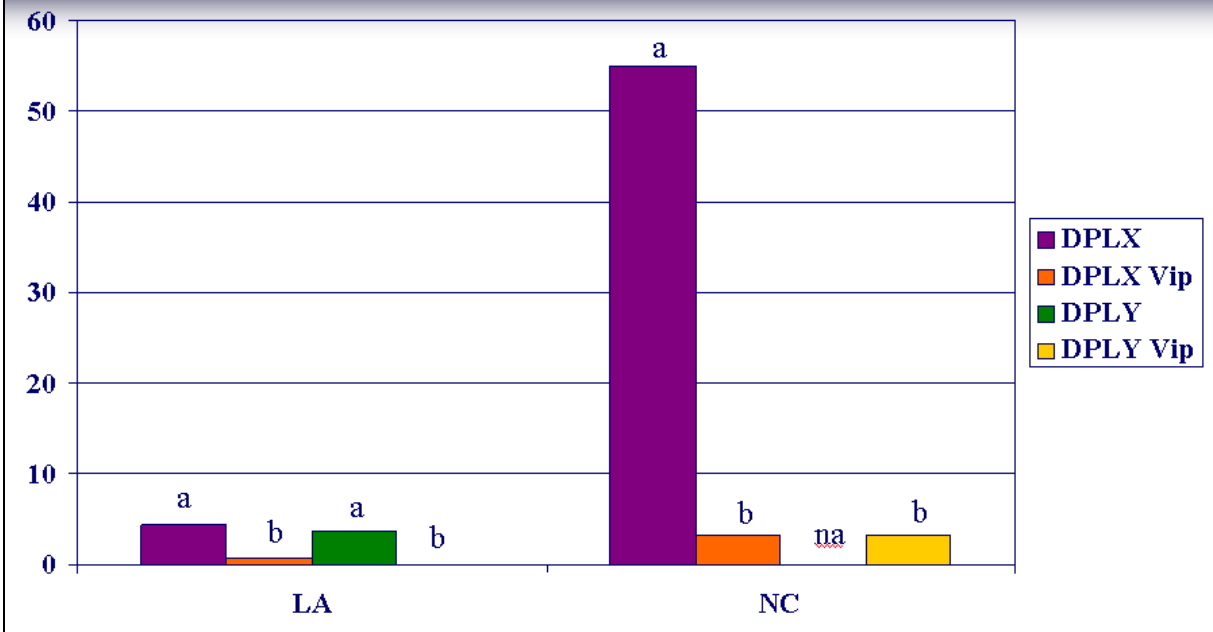
Cumulative Percent Damaged Bloom Tag



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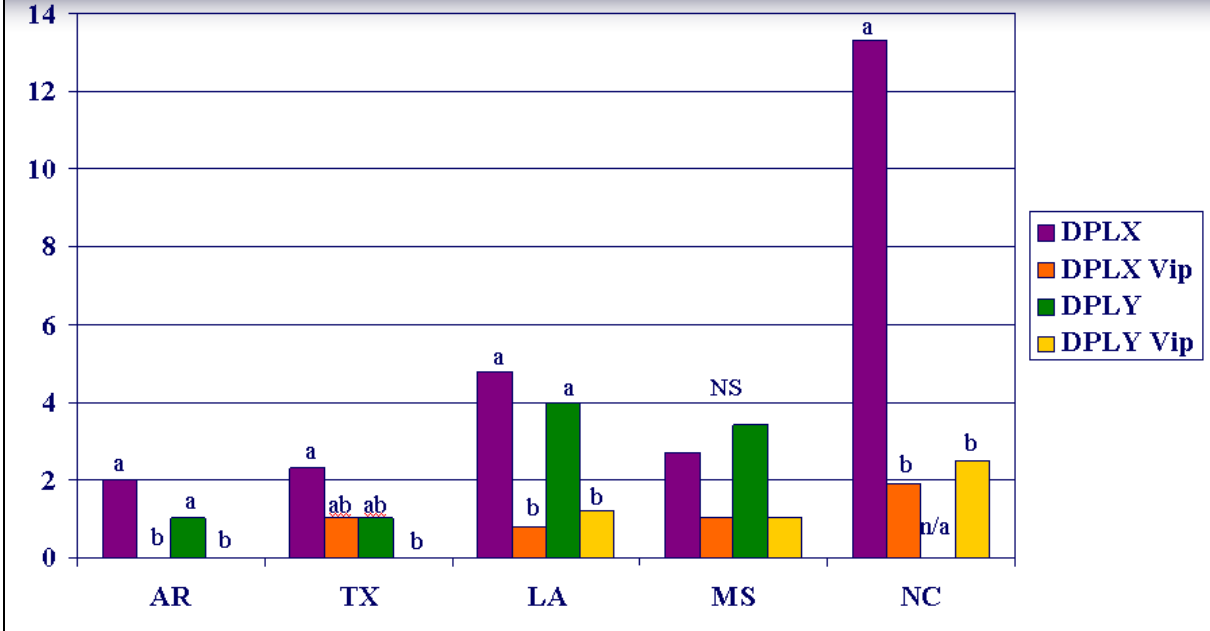
Cumulative Percent Infested Bolls



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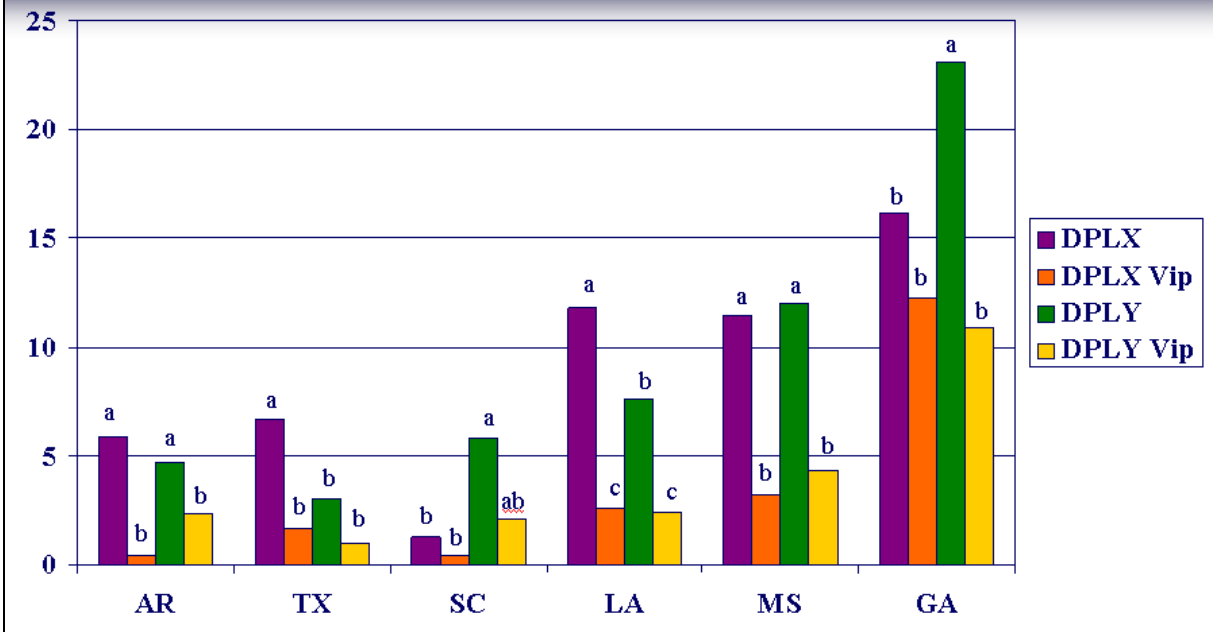
Cumulative Percent Damaged Bolls



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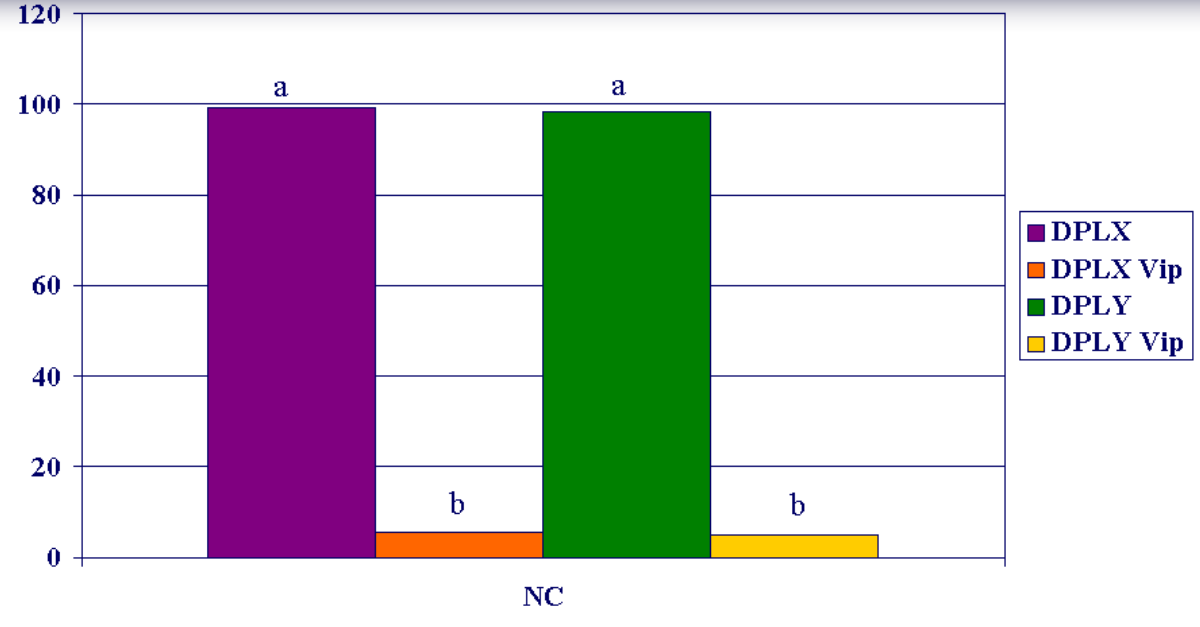
Cumulative Percent Damaged Bolls



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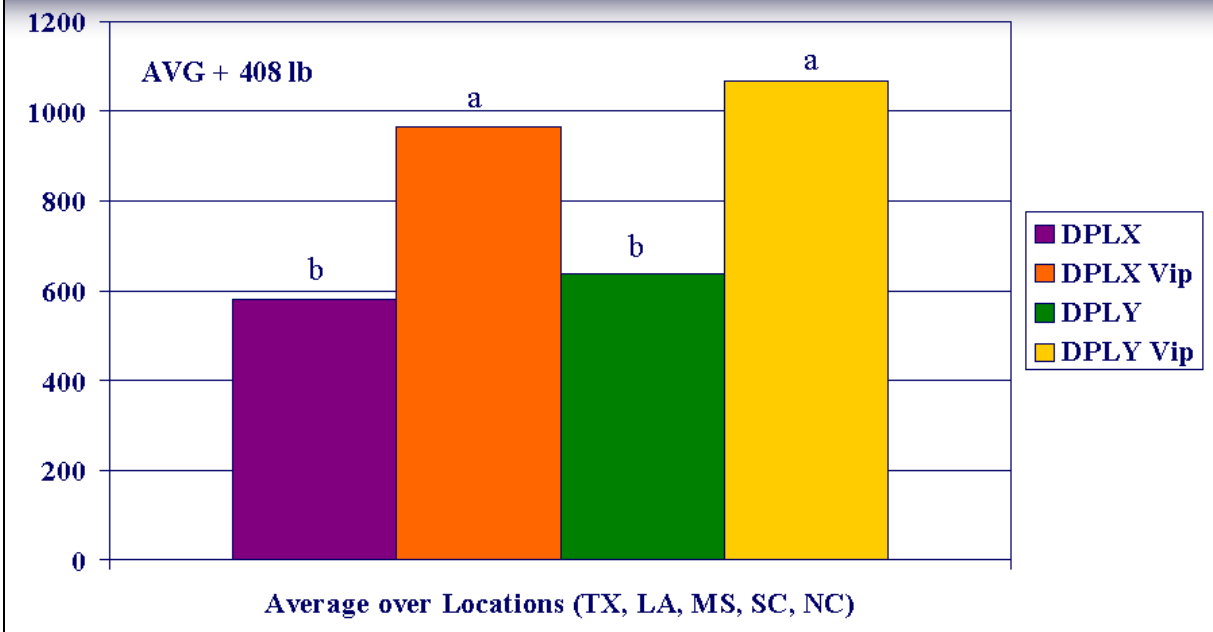
Cotton Lint (lbs)/A



Average over Locations (TX, LA, MS, SC, NC)

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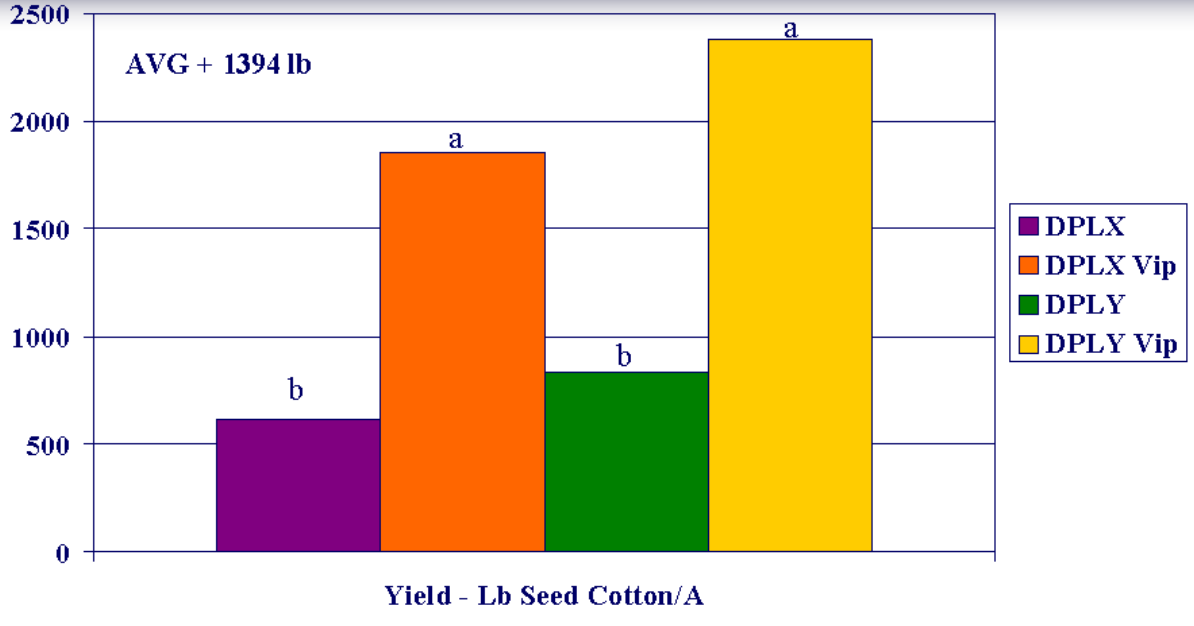
Cotton Yield - GA



Yield - Lb Seed Cotton/A

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Vip: Conclusion

- Effective control of BW/TBW complex
- Effectively reduced damage to squares and bolls
- Effective protection of apical portion of bolls covered by bloom tags indicates a robust insecticidal protein expression throughout all the plant structures critical to yield
- Novel protein represents an exciting opportunity to offer grower a new tool for insect control with unique fit in IPM programs and IRM strategies

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