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FIELD PERFORMANCE OF VIP COTTON AGAINST VARIOUS LEPIDOPTERAN COTTON PESTS IN THE U. S.

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Abstract

In 2002, Vip cotton was evaluated across the cotton belt to determine its activity against various cotton insect pests. Vip provided efficacious control of numerous lepidopteran cotton pests including the tobacco budworm (*Heliothis virescens* Fabricius), cotton bollworm (*Helicoverpa zea* Boddie), soybean looper (*Pseudoplusia includens* Walker), beet armyworm (*Spodoptera exigua* Hübner) and cotton leaf perforator (*Bucculatrix thurberiella* Busck). The insect pest protection provided by Vip resulted in significant yield increases.

Introduction

The commercialization of Bt cotton in 1996 offered growers a new tool for control of the tobacco budworm (*Heliothis virescens* Fabricius) and the cotton bollworm (*Helicoverpa zea* Boddie), two of the most devastating lepidopteran cotton pests in the mid-south and southeastern portions of the cotton belt. Adoption of Bt cotton increased rapidly throughout most of the cotton belt, from an estimated 1,851,094 acres in 1996 to 5,840,747 acres in 2001 (Williams 1997-2001). The efficacy, spectrum of activity, economic impact and resistance management strategies of Bt cotton systems have been researched extensively throughout every cotton producing region of the U. S. While expression levels of CryIAc in Bt cotton provide efficacious control of tobacco budworm, they are less than sufficient to control high populations of bollworm and often require applications of conventional insecticides to avoid yield losses (Bacheler and Mott 1997; Burd et al. 1999; Layton et al. 1997, 1998; Leonard et al. 1997, 1998). Layton et al. (2000) reported a continual increase (from 28% in 1996 to 79% in 1998) in the percentage of Mississippi's Bt cotton acreage requiring one or more insecticide applications for bollworm. Of the 407,267 cotton acres treated for the budworm/bollworm complex in Louisiana during 1999, 53.2% were Bt cotton acres (Williams 2000). These reports serve to emphasize the need of an integrated pest management strategy to effectively control the pest spectrum commonly found in cotton fields throughout the mid-south and southeastern U. S.

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). 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. 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, which have been genetically modified to express the Vip gene.

Materials and Methods

Performance of Vip cotton was evaluated across various locations throughout the cotton belt in 2002. A total of 16 internal and University cooperator trials were conducted across Texas, Louisiana, Mississippi, Arkansas, Alabama, Georgia and North Carolina. Data presented in this manuscript include trials from the following cooperators: Roy Parker - TAMU, Roger Leonard - LSU, John Ruberson and Phillip Roberts – UGA 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. Vip cotton and its parent isoline, Coker, were evaluated in side-by-side comparisons with no additional insecticide applications made for Lepidopteran control. 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 terminals, squares, flowers, 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 harvesting the center four rows of 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

Budworm and Bollworm Complex

Although some locations reported nearly discrete bollworm populations for most of the season (Waco-TX, Newport-AR, Houston Co.-AL, Jamesville, NC), many locations reported a mixture of the Heliothine complex with some locations shifting from bollworm early season to predominantly budworm during late season (Table 1). In addition, insect pressure and duration of moth flights varied widely from one location to the next.

Across all the locations in 2002, there were no differences between Vip and Coker with respect to numbers of eggs observed (Table 2), indicating no ovipositional preference by moths of the Heliothine complex. Averaged across the locations, the cumulative percent of terminals with at least one egg were 12.4 and 11.3% for Vip and Coker, respectively. However, Vip did significantly impact the survival of the developing larvae. The cumulative percent larval infestation observed in Vip terminals was significantly lower compared to Coker in five of the six locations reported (Table 3). Percent terminal infestation ranged from 0 to 4.3% in Vip compared to 1.4 to 34.5% in Coker. Averaged across the six locations, there were 6.3 times more larvae observed in Coker terminals than in Vip. In addition, Vip significantly reduced the level of damage to cotton terminals compared to Coker (Table 3). Cumulative percent damaged terminals ranged from 5 to 28.5% in Vip compared to 18 to 71.5% in Coker. Percent Heliothine larvae infestation observed in squares was significantly reduced in Vip compared with Coker (Table 4). Eight of the nine locations reported significantly lower percent square infestation in Vip (0 to 6%) compared to Coker (2.5 to 34%). Very low insect pressure in the Tift Co., GA location did not allow for statistical separation of the treatments. Averaged across all locations, Coker plots contained 6.9 times more larvae than Vip. Percent damaged squares were significantly lower in Vip than Coker in all locations (Table 4). Cumulative percent damaged squares ranged from 0 to 12.2% and 6.2 to 69.7% for Vip and Coker, respectively. Averaged across all locations, percent damaged squares in Vip were 7 times lower than in Coker. Vip expression in flowers resulted in a significant reduction in the percent larval infestation observed in this structure across most locations (Table 5). Cumulative percent flower infestation ranged from 1.5 to 20.7% in Vip compared with 6.4 to 45% in Coker. Averaged across all locations, Coker exhibited 3 times more larvae on flowers than Vip. In addition, Vip had significantly lower flower damage compared with Coker (Table 5). Cumulative percent damage flower ranged from 2.6 to 21.2% in Vip compared with 14.4 to 64% in Coker. Averaged across all locations, percent damaged flowers in Vip were 6.7 times lower than in Coker. In regards to Vip expression in bloom tags, also referred as stuck blooms, and in the apical portion of bolls covered by bloom tags, there were significantly fewer larvae and reduced damage observed in Vip plots compared with Coker (Table 6). Cumulative percent bloom tag infestation ranged from 1.4 to 2.2% and 10.9 to 15.1% for Vip and Coker, respectively. In addition, Vip plots had significantly lower cumulative percent damage to apical areas of bolls covered by bloom tags (1.6 to 3.5%) compared with Coker plots (20 to 35.9%). The cumulative percent larval infestation observed in Vip bolls was significantly lower compared to Coker in all locations reported (Table 7). Percent boll infestation ranged from 0.4 to 3% in Vip plots compared to 3.1 to 41.5% in Coker. Averaged across the six locations, there were 9.2 times more larvae observed in Coker bolls than in Vip bolls. Percent damaged bolls were significantly lower in Vip than Coker at all locations (Table 7). Cumulative percent damaged bolls ranged from 0.6 to 8.2% and 3.2 to 66.5% for Vip and Coker, respectively. Averaged across all locations, percent damaged bolls in Vip plots were 6.8 times lower than in Coker.

Beet Armyworm

Overall, beet armyworm pressure was low to non-existent in most locations in 2002. However, Vip efficacy was assessed at five locations (Table 8). In all locations, Vip plots had significantly lower numbers of larvae compared with Coker plots. Although assessment methods varied across locations, numbers of beet armyworm larvae were reduced from 89.3 to 100% in the Vip plots compared with Coker.

Soybean Looper

Although soybean looper pressure in cotton was light to moderate in most locations in 2002, efficacy was evaluated at three locations (Table 9). Vip significantly reduced the number of soybean looper larvae compared with Coker in two of the three locations. Even though assessment methods varied among locations, Vip resulted in a reduction in larval numbers ranging from 60 to 97%.

Cotton Leaf Perforator

Activity of Vip on cotton leaf perforator was only assessed at Corpus Christi in 2002. Vip significantly reduced the numbers of larvae per leaf (0.2) compared with Coker (3.0). Similarly, leaf damage observed in Vip (3.2%) was significantly less than that observed in Coker (48.7%).

Yield

Vip cotton yielded significantly more cotton than Coker in eight of the ten locations reported (Table 10). Environmental conditions and/or lack of sufficient insect pressure were the factors mostly responsible for the inability to detect significant yield differences between Vip and Coker in the two Georgia locations. In the Brooks, GA location, prolonged rainfall prevented timely harvest of plots resulting in excessive lint drop. In the Tift Co., GA location, light insect pressure coupled by optimal growing conditions late in the growing season allowed Coker plants to set a significant number of second and third position bolls, resulting in a compensatory effect. Box mapping data (data not shown) demonstrated substantially fewer first position bolls in Coker compared to Vip (Phillips Roberts, personal communication). Vip cotton yields ranged from 1,247 to 2,629 lb seed cotton per acre compared with 459 to 1,605 lb seed cotton per acre for Coker. Averaged across all locations, yields for Vip and Coker were 1,991 and 1,024 lb, respectively. These differences represent an average increase of 967 lb of seed cotton per acre due to Vip.

Discussion

Whether tested against discrete bollworm populations, or a bollworm/budworm pest complex, Vip cotton provided effective season long control of these pests. Vip effectively reduced the level of damage to squares, flowers and bolls by an average of 85% compared to Coker, indicating a robust insecticidal protein expression throughout the plant structures critical to yield and those that are commonly attacked by the Heliothine complex. In addition, Vip expression in the apical portion of bolls covered by bloom tags resulted in a 91% reduction in damage compared to Coker. Although limited field data is available on the efficacy of Vip cotton against other lepidopteran pests, results presented here indicate that Vip cotton is highly efficacious on beet armyworm, soybean looper and cotton leaf perforator. Vip's efficacy against the Heliothine complex and broad spectrum of activity against other lepidopteran pests makes it a very attractive tool for the control of key cotton pests. In addition, Vip represents a novel insecticidal gene that is structurally distinct, with a different mode of action from delta-endotoxin proteins, which are currently marketed. These attributes enable Vip to have a unique fit into IPM systems in cotton, as well as resistance management strategies for all Bt derived insecticidal proteins.

References

Bacheler, J.S., and D.W. Mott. 1997. Efficacy of grower managed Bt cotton in North Carolina, pp. 858-861. *In* P. Dugger and D. A. Richter [eds.], Proceedings, 1997 Beltwide Cotton Conference. National Cotton Council, Memphis, TN.

Burd, T., J.R. Bradley, Jr., and J.W. Van Duyn. 1999. Performance of selected Bt cotton genotypes against bollworm in North Carolina, pp. 931-934. *In* P. Dugger and D.A. Richter [eds.], Proceedings 1999 Beltwide Cotton Conference. National Cotton Council, Memphis, TN.

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.

Layton, M.B, M.R. Williams, and S. Stewart. 1997. Bt cotton in Mississippi: the first year, pp. 861-863. *In* P. Dugger and D.A. Richter [eds.], Proceedings, 1997 Beltwide Cotton Conference. National Cotton Council, Memphis, TN.

Layton, M.B., S. D. Stewart, and M.R. Williams. 1998. Performance of Bt cotton in Mississippi, 1997, pp. 970-973. In P. Dugger and D.A. Richter [eds.], Proceedings, 1998 Beltwide Cotton Conference. National Cotton Council, Memphis, TN.

Layton, M.B., M.R. Williams, and J.L. Long. 2000. Performance of Bt cotton in Mississippi, 1999, pp. 1037-1039. In P. Dugger and D.A. Richter [eds.], Proceedings, 2000 Beltwide Cotton Conference. National Cotton Council, Memphis, TN.

Leonard, B.R., J.H. Fife, K. Torrey, J.B. Graves, and J. Holloway. 1997. Helicoverpa/Heliothis management in Nu-COTN and conventional cotton cultivars in Louisiana, pp. 863-867. In P. Dugger and D.A. Richter [eds], Proceedings, 1997 Beltwide Cotton Council, Memphis, TN.

Leonard, B.R., J.H. Fife, K. Torrey, E. Burris, and J.B. Graves. 1998. Evaluation of transgenic Bt cotton lines against Heliothines in Northeast Louisiana, pp. 967-970. *In* P. Dugger and D.A. Richter [eds.], Proceedings, 1998 Beltwide Cotton Conference. National Cotton Council, Memphis, TN.

Williams, M.L. 1997. Cotton insect losses, pp. 834-853. *In* P Dugger and D. A. Richter [eds.], Proceedings, 1997 Beltwide Cotton Conference. National Cotton Council, Memphis, TN.

Williams, M.L. 1998. Cotton insect losses, pp. 904-925. *In* P Dugger and D. A. Richter [eds.], Proceedings, 1998 Beltwide Cotton Conference. National Cotton Council, Memphis, TN.

Williams, M.L. 1999. Cotton insect losses, pp. 785-806. *In* P Dugger and D. A. Richter [eds.], Proceedings, 1999 Beltwide Cotton Conference. National Cotton Council, Memphis, TN.

Williams, M.L. 2000. Cotton insect losses, pp. 887-913. *In* P Dugger and D. A. Richter [eds.], Proceedings, 2000 Beltwide Cotton Conference. National Cotton Council, Memphis, TN.

Williams, M.L. 2001. Cotton insect losses. *In* P Dugger and D. A. Richter [eds.], Proceedings, 2000 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).