

FIELD PERFORMANCE OF A VIPCOT COTTON LINE AGAINST HELIOTHINES IN LOUISIANA

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Abstract

A series of field studies during 2005-2007 evaluated the performance of transgenic cotton expressing the Vip3A insecticidal protein against native and artificial infestations of bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.). The conventional non-Bt cotton cultivar, 'Coker 312', and two *Bacillus thuringiensis* (Bt) cotton lines expressing either a single protein (Vip3A) or combination of proteins (Vip3A + Cry1Ab [VipCot™]) were sampled throughout the 2005-2007 production seasons for larval injury to fruiting forms and surviving larvae. Species composition and levels of native infestations varied within a season and across seasons, but both Bt cotton lines had significantly fewer damaged fruiting forms and surviving larvae compared to those found on Coker 312 plants. VipCot™ cotton plants had lower numbers damaged fruiting forms and fruiting forms infested with larvae compared to that on Vip3A plants. In addition, selected Coker 312, Vip3A, and VipCot™ plants in field plots were infested with either bollworm or tobacco budworm larvae. These plants were visually inspected at 3 d after infestation and every 2 d thereafter, until larvae were no longer detected. Lower levels of damaged fruiting forms and fewer larvae for both species were recorded on Vip3A, and VipCot™ plants compared to those on Coker 312 plants. A bollworm larva injured an average of 8.6, 4.6, and 1.0 fruiting forms on Coker 312, Vip3A, and VipCot™ plants, respectively. A tobacco budworm larva injured an average of 9.2, 5.9, and 0.9 fruiting forms on Coker 312, Vip3A, and VipCot™ plants, respectively. The patterns of seasonal efficacy generally showed the VipCot™ plants to be more durable with less fruiting form injury than that recorded on Coker 312 and Vip3A, especially during periods of peak heliothine infestations. The combination of two insecticidal proteins expressed in the VipCot™ cotton line improved efficacy levels against a complex of heliothines above that of the single protein in the Vip3A line.

Introduction

Transgenic cottons, which express proteins from the soil bacterium, *Bacillus thuringiensis*, are the standard management strategy for primary lepidopteran pests. The first commercial transgenic Bt cotton, Bollgard® provides excellent control of tobacco budworm, *Heliothis virescens* (F.), but bollworm, *Helicoverpa zea* (Boddie) control has not been consistent. Bollworms are inherently less toxic to the Cry1Ac protein in Bollgard than tobacco budworm (Luttrell et al. 1999). Also, Cry1Ac levels decrease as the plant ages (Greenplate 1999, Adamczyk et al. 2001, Oslen et al. 2005) and vegetative tissue of Bollgard® plants expresses higher cry protein levels compared to that in floral structures such as pollen and flower petals (Greenplate 1999, Adamczyk et al. 2001, Gore et al., 2001). Bollworm larvae are often observed feeding in flowers of Bollgard® plants which can result in relatively high levels (more than 50%) of boll abscission (Smith 1998, Gore et al. 2000).

The inconsistent efficacy of Bollgard® against bollworm and other occasional lepidopteran pests prompted the agrochemical companies to develop more broad spectrum transgenic technologies. Therefore, in recent years, cotton cultivars that express two insecticidal proteins (Bollgard II® and WideStrike®) were commercialized. These products provide season long, broad spectrum control of major lepidopteran pests above that provided by the single protein

expressed in Bollgard® Plants. Several studies with Bollgard II® and WideStrike® have demonstrated significantly better control of bollworms and other lepidopteran pests than Bollgard® cotton (Gore et al. 2001, Stewart et al. 2001, Leonard et al. 2005, Willrich et al. 2005).

Novel transgenic cotton plants which express a vegetative insecticidal protein, Vip3A, are being developed by Syngenta Crop Protection (Lee et al. 2003). This insect pest management technology has been trademarked VipCot™ and includes cotton lines that have been transformed to express both Vip3A and Cry1Ab proteins (McCaffery et al. 2006). The Vip3A is different from Cry proteins found in all current commercial products (Bollgard®, Bollgard II®, and WideStrike®). The insecticidal Cry proteins are produced during the bacterium reproductive phase, enclosed in crystals, and classified as endotoxins. Vip3A is secreted during the vegetative phase of bacterium development, and considered an exotoxin (Micinski and Waltman 2005, Yu et al. 1997).

Relatively few field studies have evaluated the efficacy of the VipCot™ technology against native and artificial infestations of heliothines and examined the seasonal durability against these lepidopteran targets. Before the VipCot™ technology can be fully integrated into a cotton pest management system; the consistency of performance against the primary heliothine targets should be documented. Therefore, the objective of this study was to evaluate the seasonal efficacy of VipCot™ cotton lines against bollworm and tobacco budworm. A second objective was to compare heliothine injury levels among fruiting structures of Coker 312, Vip3A, and VipCot™ cotton lines.

Materials and Methods

Native Infestations of Heliothines in Field Trials. These studies were performed at the Louisiana State University Agricultural Center's Macon Ridge Research Station near Winnsboro, LA (Franklin Parish) from 2005 to 2007. The conventional non-Bt cotton cultivar, 'Coker 312', and Bt cotton lines expressing either a single protein (Vip3A) or combination of proteins (Vip3A + Cry1Ab [VipCot™]) were planted in a Gigger-Gilbert silt loam soil on 8 Jun in 2005, on 20 Jun in 2006, and on 16 Jun in 2007. Normal cultural practices and integrated pest management strategies recommended by the Louisiana Cooperative Extension Service were used to optimize plant development across the test site (Bagwell et al. 2005, Stewart et al. 2007). The plots in these studies were not treated with any insecticides specifically for heliothine control. However, Chemical control of non-target pests such as thrips, *Frankliniella* spp., cotton aphids, *Aphis gossypii* (Glover), and tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois) during the study was accomplished with insecticides aldicarb (Temik 15G, Bayer CropScience, Research Triangle Park, NC), dicotophos (Bidrin 8EC, Amvac Chemical Corporation, Los Angeles, CA), imidacloprid (Trimax SC, Bayer CropScience, Research Triangle Park, NC), and thiamethoxam (Centric 40 WG, Syngenta Crop Protection, Greensboro, NC) which express minimum efficacy against heliothines.

Cotton lines were evaluated by examining 25 randomly selected fruiting forms, flower buds (squares) and bolls, from the two center rows of each plot for incidence of heliothine damage and surviving larvae. Plots were sampled once to twice weekly from ~40 days after planting to 100 days after planting (mid-Jul to late Sept). Species composition across the test areas was estimated with pheromone trap captures of heliothine adults. In addition, collections of larvae were examined from adjacent plots (border rows) of non-Bt cotton to further support the seasonal trap capture data.

Treatments (cotton lines) were arranged in a randomized block design with four replications. The analysis was standardized to include in only those dates on which average fruiting form injury was $\geq 5\%$ in the Coker 312 plots. The number of sample dates included in the analysis during 2005, 2006, and 2007 were 6, 10, and 9, respectively. The numbers of damaged fruiting forms and surviving heliothine larvae were converted to percentages, averaged across all samples and then subjected to ANOVA using PROC MIXED (ver. 9.1; SAS Institute; Cary, NC). The seasonal durability data was subjected to a two-way factorial analysis with cotton line and days after planting as factors.

Artificial Infestations of Heliothines in Field Trials. These experiments also were performed at the Louisiana State University Agricultural Center Macon Ridge Research Station near Winnsboro, LA (Franklin Parish) in 2006. The non-Bt cotton cultivar, Coker 312, and Bt cotton lines Vip3A and VipCot™ were planted in a Gigger-Gilbert silt loam soil on 15 Jun, 2006. The cotton lines were arranged in a completely randomized design across the test area.

The test area was maintained with agronomic and pest management practices in a manner similar to that previously described.

The procedures for the artificial infestation study followed the general outline described by Bommireddy et al. (2007). A colony of *H. zea* and *H. virescens* was established from sweet corn, *Zea mays* L., and garbanzo beans, *Cicer arietinum* L., respectively during June, 2006. Insects were reared in the laboratory for a minimum of one generation to eliminate parasitoids and pathogens and to obtain sufficient numbers of larvae. All infestations on the cotton lines were completed within two generations of removal from the field.

Field plots of Coker 312, Vip3A, and VipCot™ cotton lines were thinned to 3 plants per meter (one plant per row-foot) before infestation to prevent interplant movement of larvae. Infestations were initiated when cotton plants across the test area had seven to nine main stem nodes above the upper-most first position white flower on a sympodial branch. All plants were in similar stages of plant development during this study. Those plants designated for infestation were examined for the presence of heliothine eggs and larvae. Only those plants without a native heliothine infestation were used in these studies. White flowers were selected for infestation and tagged with a yellow snap on tag (A. M. Leonard, Inc., Piqua, OH). A single L2 stage heliothine larva (72 ± 6 h old) was placed in a first position white flower on a single plant of each cotton line using a small camel's hair brush. Twenty five plants of the Coker 312, Vip3A, and VipCot™ cotton lines were independently infested with each species on each of three days.

The infested plants were visually inspected at 3 d after infestation for damage to the fruiting structure at the infested site and for the presence of surviving larvae. Thereafter, entire plants were inspected every 2 d for cumulative damage to fruiting structures (squares, white flowers, and bolls) until larvae were no longer detected. Non-infested plants adjacent to the infested plants were monitored for natural abscission of fruiting structures due to native Heliothine populations. The effects of native heliothines during this period were suppressed by removing and destroying any eggs or small larvae not associated with the experiment. Numbers of damaged fruiting forms and surviving larvae were recorded from the same experimental units over independent rating intervals during the study; therefore, these data were subjected to a repeated measures ANOVA (PROC MIXED, ver. 9.1; SAS Institute; Cary, NC). The total number of fruiting forms damaged by an individual larva for each species was subjected to ANOVA (PROC MIXED ver. 9.1; SAS Institute; Cary, NC).

Results

Native Infestations of Heliothines in Field Trials. Pheromone trap captures and samples of larvae collected from non-Bt cotton plants adjacent to the test areas indicated that the bollworm was the most common species (>80% seasonal composition) infesting plants during all three years. Populations of tobacco budworm were considerably lower than bollworm during each year, and this species was only common during the late season (81-100 DAP).

The number of fruiting forms damaged by heliothines was significantly higher in Coker 312 compared to Vip3A and VipCot™ cotton lines (Table 1). VipCot™ cotton had significantly fewer heliothine damaged fruiting forms compared to Vip3A cotton. Fruiting forms infested with surviving larvae were also significantly influenced by cotton type. Larval numbers were higher on Coker 312 compared to that on Vip3A and VipCot™ cotton plants. The VipCot™ plants had significantly fewer heliothine larvae compared to that on Vip3A cotton plants.

Heliothines damaged significantly more fruiting forms in Coker 312 plots compared to Vip3A and VipCot™ plots during the period of 40-90 days after planting (DAP) (Fig. 1). A single defined peak was observed in heliothine damaged fruiting forms on Coker 312 and Vip3A plots at 71-80 DAP. During this period, the number of damaged fruiting forms was 7.8, 2.6, and 0.4 per 25 plants in Coker 312, Vip3A, and VipCot™ cotton, respectively. In addition, this was the only period of time when a significant difference in damaged fruiting forms was detected between Vip3A and VipCot™ plants. The pheromone trap captures and samples of larvae from adjacent non-Bt plots indicated that the bollworm (>70%) was still the dominant species. However, low numbers of tobacco budworm were detected and began to increase during the 71-80 DAP period. Tobacco budworm did not become the dominant species ($\approx 65\%$) until overall heliothine populations declined at 81-100 DAP.

Artificial Infestations of Heliothines in Field Trials. Bollworm larvae injured more fruiting forms on Coker 312 compared to that on Vip3A and VipCot™ cotton plants at all rating intervals (Fig. 2). Cumulative injury to fruiting forms also was significantly higher on Vip3A cotton compared to VipCot™ cotton at 3, 5, 7, and 9 d after infestation.

Significantly more bollworm larvae were recorded on Coker 312 plants compared to that on both Bt cotton lines at all rating intervals (Fig. 2B). At 3, 5, and 7 d after infestation, fewer surviving bollworm larvae were detected on VipCot™ plants compared to that on Vip3A plants. By 7 d, no larvae were recorded on VipCot™ plants, but larvae were still found on Coker 312 and Vip3A plants. No bollworm larvae were found on Vip3A plants at 9 d after infestation, but 8.3 larvae per 25 plants were recorded on the Coker 312 cotton line.

A bollworm larva injured more squares, flowers, and bolls on Coker 312 than on Vip3A and VipCot™ cotton lines (Fig. 3). An average of 8.6 fruiting forms (2.6 squares, 2.3 white flowers, and 3.5 bolls) on Coker 312 plants were injured per bollworm larva. On Vip3A plants, a bollworm larva injured 4.6 fruiting forms (2.1 squares, 0.5 white flowers, and 1.9 bolls). Although VipCot™ plants were damaged less by bollworm than Vip3A plants, a low level of fruiting form injury was recorded. A bollworm larva damaged an average of 1.0 fruiting forms (0.6 squares, 0.2 white flowers, and 0.2 bolls) during the 9 d evaluation period.

Tobacco budworm larvae injured significantly more fruiting forms on Coker 312 plants compared to that on Vip3A and VipCot™ plants at all rating intervals (Fig. 4A). Cumulative injury to fruiting forms also was significantly higher on Vip3A cotton compared to VipCot™ cotton at 3, 5, 7, and 9 d after infestation.

Significantly more tobacco budworm larvae were recorded on Coker 312 compared to both Bt cotton lines at all rating intervals (Fig. 4B). In addition, fewer surviving tobacco budworm larvae were detected on VipCot™ plants compared to that on Vip3A plants at all rating intervals. At 7 d, no larvae were found on VipCot™ plants, but 13.3 and 4.7 larvae per 25 plants were recorded on Coker 312 and Vip3A plants. No larvae were found on Vip3A plants by 9 d after infestation.

A tobacco budworm larva injured more squares, flowers, and bolls on Coker 312 than on Vip3A and VipCot™ cotton lines (Fig. 5). On Coker 312 plants, a larva damaged 9.2 fruiting forms (2.6 squares, 3.3 white flowers, and 3.2 bolls). A larva damaged 5.9 fruiting forms (2.3 squares, 0.5 white flowers, and 3.0 bolls) on Vip3A plants, as observed with bollworm, total fruiting form injury by tobacco budworm was lower on VipCot™ plants compared to that on Vip3A plants. A tobacco budworm larva injured 0.9 fruiting forms (0.4 squares, 0.2 white flowers, and 0.3 bolls) on VipCot™ plants.

Discussion

Bollworm and tobacco budworm injured more fruiting forms on Coker 312 plants compared to that on the single protein, Vip3A, and stacked proteins, VipCot™ plants during the native and artificial infestation studies. Fruiting forms infested with surviving larvae also were lower on plants of both Bt cotton lines compared to that on Coker 312 plants. The VipCot™ line generally sustained significantly less injury to fruiting forms and maintained a lower larval infestation compared to that on the Vip3A cotton line. In field trials evaluating the performance of single and stacked Cry proteins expressed in cotton lines, Jackson et al. (2003) reported patterns of efficacy against bollworm similar to that presented in the present study. Bollgard® plants expressing a single cry protein (Cry 1Ac) had more squares (4.6%) and bolls (9.3%) damaged than squares (1.8%) and bolls (1.3%) of Bollgard II® plants expressing two cry proteins (Cry1Ac + Cry 2Ab). Fruiting forms infested with larvae ranged from 0.9 to 2.9% on Bollgard® plants and 0.3 to 0.5% on Bollgard II® plants. Adamczyk et al. (2001) found significantly fewer damaged squares (0.7) on Bollgard II® plants compared to those on Bollgard® (6.2%) and non-Bt (7.7) plants. This reduction in fruiting form injury and larval survival on Bollgard II® plants compared to that on Bollgard® plants is directly related to the effects generated by the second protein (Cry2Ab). The combination of two proteins in Bollgard II® has increased activity against several lepidopteran target pests (Adamczyk et al., 2001; Jackson et al. 2003). In the present study, the combining effects of the Cry1Ab protein to that of Vip3A enhanced the overall efficacy of the VipCot™ line against heliothines compared to that for the Vip3A line. The results of limited field trials also have demonstrated that VipCot™ cotton lines have provided satisfactory control of heliothines (Leonard et al. 2005, Micinski and Waltman, 2005, Parker and Livingston 2005).

In addition, profiling the seasonal distribution of fruiting form damage indicates that the VipCot™ line sustained less injury during the heliothine peak infestation period (71-80 DAP) compared to injury to Vip3A and Coker 312 fruiting forms. During that period of peak infestation, bollworm was the dominant species, but low levels of tobacco budworm were present. Wan et al. (2005) also documented lower *Helicoverpa armigera* (Hubner) larval densities on Bt cotton lines GK19 (Cry1Ac+ Cry1Ab) and BG1560 (Cry1Ac) throughout the season compared to that on non-Bt cotton. For many of the commercial Bt cotton lines expressing Cry proteins, overall levels decrease as the plant ages during the season (Greenplate et al. 1999). Furthermore, a decline in efficacy of cry proteins in Bt cottons against *H. armigera* has been observed in Australia (Fitt et al. 1998). In the present study, the efficacy of the Vip3A line was not as consistent as that in the VipCot™ during the season. This observation may be related to several of factors such as species selectivity, infestation pressure, plant genotype and environment interaction as well as a seasonal decline in protein expression.

The artificial infestation study isolated injury to individual fruiting forms and species specific survivorship of larvae. The conventional non-Bt, Coker 312 plants sustained significantly higher square damage at all rating intervals compared to that on Vip3A and VipCot™ plants, regardless of heliothine species. Bollworm-damaged fruiting forms ranged from 23.0-44.1, 7.0-21.1, and 2.7-6.0 per 25 plants on Coker 312, Vip3A, and VipCot™ plants, respectively. However, tobacco budworm injured 21.7-50.7, 9.3-23.3, and 2.6-4.7 fruiting forms per 25 plants on Coker 312, Vip3A, and VipCot™ cotton, respectively. Though injury on Vip3A cotton was significantly lower compared to that on Coker 312 plants, significant numbers of damaged fruiting forms were observed on Vip3A plants for bollworm and tobacco budworm. In addition, heliothine injury on VipCot™ cotton was significantly low at all rating intervals. Studies evaluating the efficacy of Bollgard® and Bollgard II® against bollworm have shown little injury on Bollgard II® compared to that on Bollgard®. Bollworm larvae injured a total of 25.0, 11.5, and 6.4 fruiting forms per 10 plants on non-Bt, Bollgard®, and Bollgard II® cotton plants, respectively, at 11 d after infestation (Gore et al. 2003).

In the present study, significantly more larvae were recorded on Coker 312 plants compared to that on Vip3A and VipCot™ plants. Similar to the results for injury to fruiting forms, significantly fewer larvae were recorded on VipCot™ plants compared to that on Vip3A plants for bollworm and tobacco budworm. No larvae of either species were found on VipCot™ and Vip3A plants at 7 and 9 d after infestation, respectively. A similar pattern of results has been observed with bollworm survivorship on Bollgard® and Bollgard II® plants. Significantly fewer bollworm larvae were recovered on Bollgard II® (25.0, < 10.0, and 0.0%) compared to Bollgard® (73.6, 59.7, and 40.3%) at 5, 7, and 9 d after infestation, respectively (Gore et al. 2003). No bollworm larvae were found on Bollgard® and Bollgard II® plants beyond 9 d after infestation.

Defining the amount and type of cotton fruiting form injury produced by an individual larva can be necessary information for ultimately establishing economic injury levels. The results of the present study for bollworm and tobacco budworm injury to fruiting forms on the non-Bt Coker 312 plants are similar to that of a number of previous studies. A bollworm and tobacco budworm larva was found to injure an average of 8.6 and 9.2 fruiting forms, respectively, on Coker 312 plants at 9 d after infestation. Studies in Arkansas found that an individual bollworm larva injured 6.0 fruiting forms (Anonymous 1967). Finally, a single tobacco budworm was capable of damaging 12.1 fruiting forms during complete larval development (Heilman et al. 1981).

Fewer studies have examined the relationship of individual larval feeding and fruiting form injury on transgenic Bt plants. This results of the present study showed that for bollworm and tobacco budworm, an individual larva injured more fruiting forms on Coker 312 compared to that on Vip3A and VipCot™ cotton. A bollworm larva injured only 4.6 and 1.0 fruiting forms on Vip3A and VipCot™ plants, respectively at 9 d after infestation. Gore et al. (2003) found that an individual bollworm larva injured an average of 6.6 fruiting forms on non-Bt cotton, 3.5 fruiting forms on Bollgard®, and 0.8 fruiting forms on Bollgard II® plants at 11 d after infestation. The results for tobacco budworm are similar those previously mentioned for bollworm. A single larva was found to injure only 5.9 and 0.8 fruiting forms on Vip3A and VipCot™ plants, respectively.

These results suggest that both species of heliothine larvae were extremely susceptible to the VipCot™ technology. None of the larvae for either species produced significant injury to fruiting forms in the field trials. In addition, none of the larvae were capable of completing larval development on the VipCot™ cotton line. However, a significant level of fruiting form injury was observed on Vip3A plants. In the artificial infestations, mortality of tobacco budworm was slower on Vip3A plants compared to that of bollworm and suggests differential susceptibility between

species. The ability of the VipCot™ technology to sustain minimal injury against both species of heliothines should allow this technology to become another useful tool for the cotton industry. To ensure season-long expression and efficacy against heliothine target pests, future work should focus on profiling the seasonal expression of Vip3A and cry protein combination on the final lines released for commercialization and among plant structures. Additional studies also need to evaluate the efficacy of VipCot™ line against a multitude of lepidopteran target pests.

Acknowledgement

The assistance of numerous student workers at the Macon Ridge Research Station in rearing insects, field plot maintenance, and infestation procedures is appreciated. We also thank the LSU AgCenter, Cotton Incorporated, and Louisiana's cotton producers for their financial support of the project. The authors acknowledge Scott Martin and David Dickerson of Syngenta Crop Protection for providing the cotton strains evaluated in these tests.

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Table 1. Seasonal (mean \pm SE) percentage of fruiting forms damaged by heliothines (bollworm, *Helicoverpa zea* [Boddie]; and tobacco budworm, *Heliothis virescens* [F.]) and infested with larvae for non-*Bacillus thuringiensis* (Bt), Coker 312, and transgenic Bt cotton, Vip3A and VipCot™ lines in Louisiana field trials during 2005-2007.

Cotton lines	Percent	
	Damaged forms ¹	Surviving larvae ¹
Coker 312	14.15 \pm 0.6a	4.61 \pm 0.4a
Vip3A	4.05 \pm 0.5b	1.02 \pm 0.2b
VipCot	0.93 \pm 0.2c	0.12 \pm 0.0c

Means within a column followed by same letter are not significantly different according to Fisher's protected LSD ($\alpha = 0.05$).

¹ Field trials sampled 6, 10, and 9 times during 2005, 2006, and 2007, respectively.

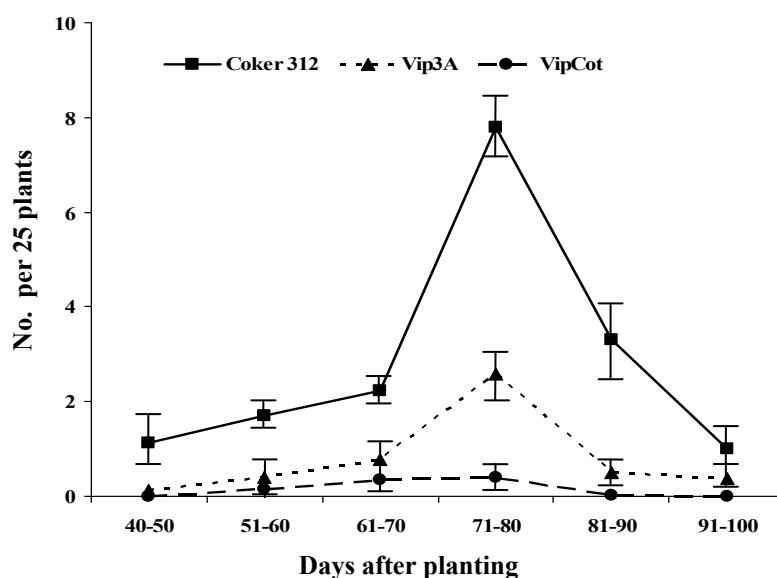


Figure 1. Seasonal distribution (mean \pm SE) of bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), damaged fruiting forms on non-*Bacillus thuringiensis* (Bt), Coker 312, and transgenic Bt cotton, Vip3A and VipCot™ lines in Louisiana field trials, 2005-2007.

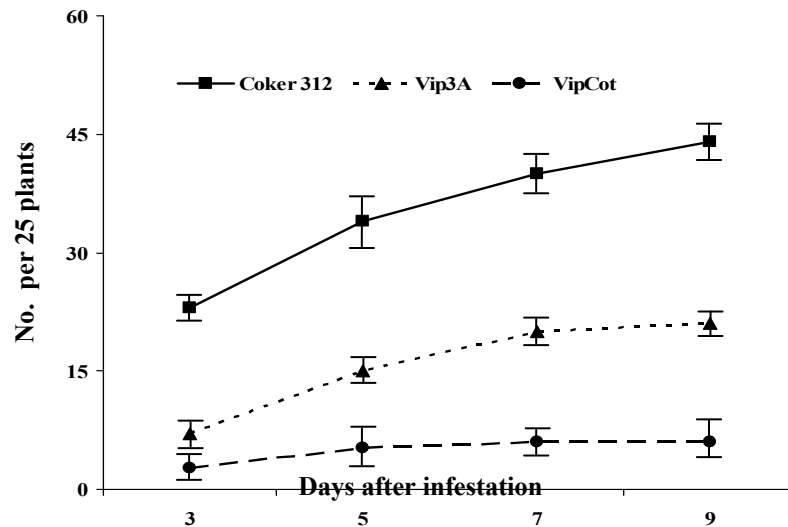


Figure 2A. Bollworm, *Helicoverpa zea* (Boddie), damaged fruiting forms (mean \pm SE) on non-*Bacillus thuringiensis* (Bt), Coker 312, and transgenic Bt cotton, Vip3A and VipCot™ lines.

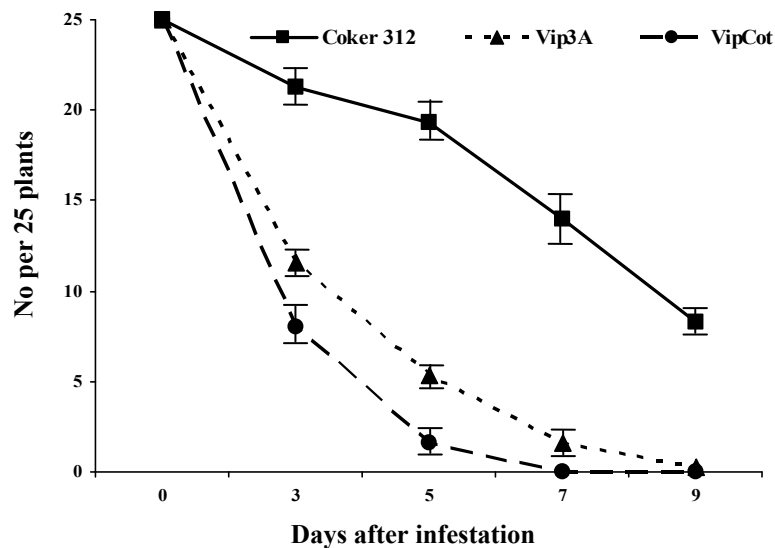


Figure 2B. Surviving bollworm, *Helicoverpa zea* (Boddie), larvae (mean \pm SE) recovered on non-*Bacillus thuringiensis* (Bt), Coker 312, and transgenic Bt cotton, Vip3A and VipCot™ lines.

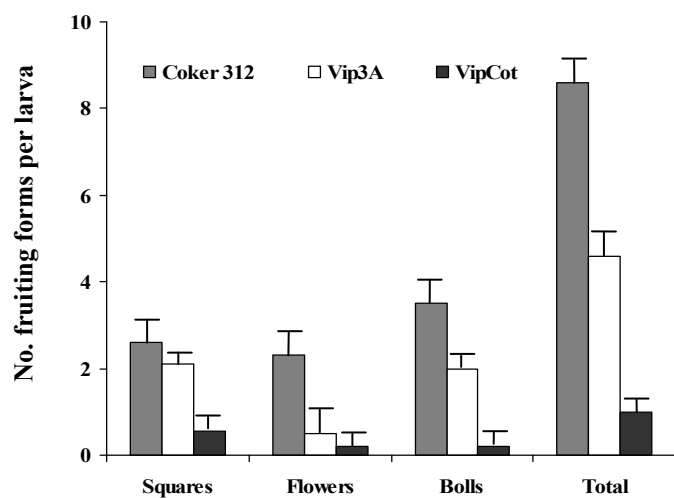


Figure 3. Bollworm, *Helicoverpa zea* (Boddie), injury to fruiting forms on non-*Bacillus thuringiensis* (Bt), Coker 312, and transgenic Bt cotton, Vip3A and VipCotTM lines at 9 d after inoculation in white flowers (bars represent mean \pm SE of structures damaged by a single larva)

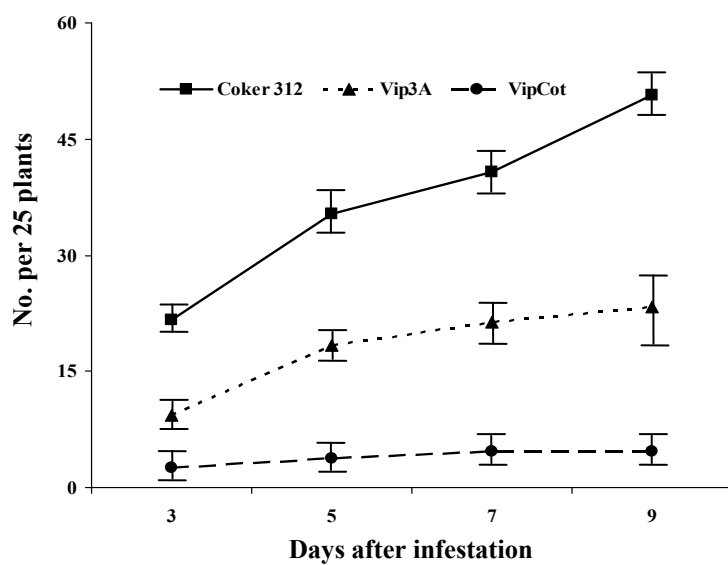


Figure 4A. Tobacco budworm, *Heliothis virescens* (F.), damaged fruiting forms (mean \pm SE) on non-*Bacillus thuringiensis* (Bt), Coker 312, and transgenic Bt cotton, Vip3A and VipCotTM lines.

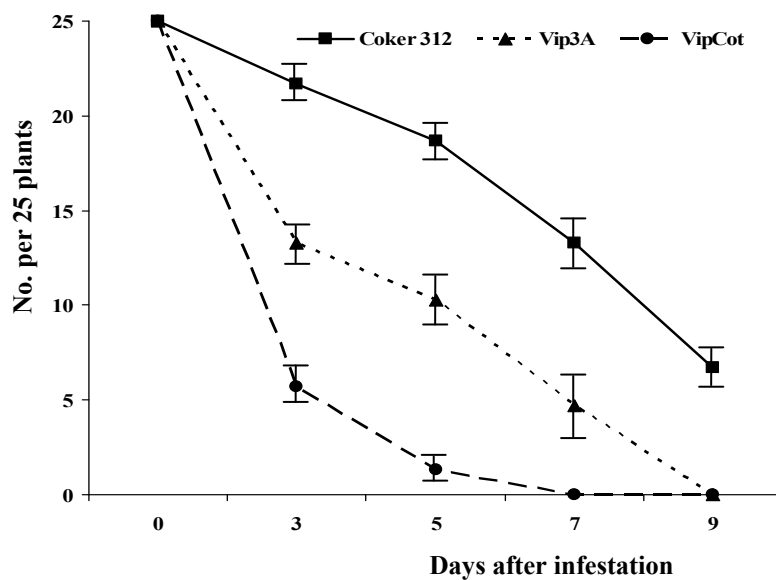


Figure 4B. Surviving tobacco budworm, *Heliothis virescens* (F.), larvae (mean \pm SE) recovered on non-*Bacillus thuringiensis* (Bt), Coker 312, and transgenic Bt cotton, Vip3A and VipCotTM lines.

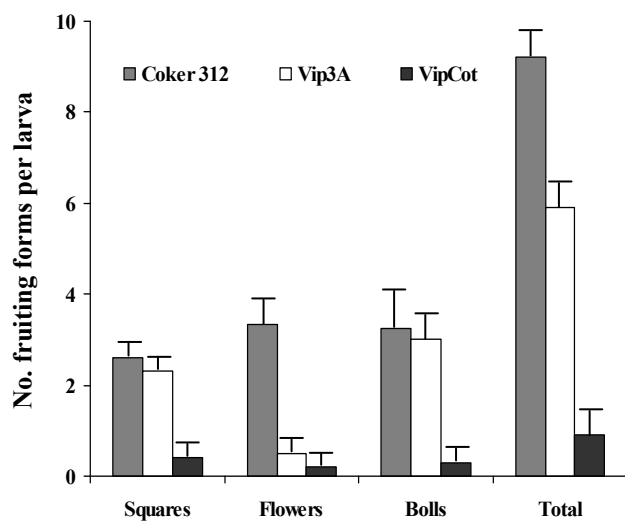


Figure 5. Tobacco budworm, *Heliothis virescens* (F.), injury to fruiting forms on non-*Bacillus thuringiensis* (Bt), Coker 312, and transgenic Bt cotton, Vip3A and VipCotTM lines at 9 d after inoculation in white flowers (bars represent mean \pm SE of structures damaged by a single larva).