

## ARTHROPOD MANAGEMENT

### Field Performance and Seasonal Efficacy Profiles of Transgenic Cotton Lines Expressing Vip3A and VipCot Against *Helicoverpa zea* (Boddie) and *Heliothis virescens* (F.)

Padma L. Bommireddy\*, B. Rogers Leonard, Joshua Temple, Paul Price, Karla Emfinger, Donald Cook, and Jarrod T. Hardke

#### ABSTRACT

A series of field trials from 2005 to 2007 evaluated the performance of transgenic cotton lines expressing the Vip3A protein against native and artificial infestations of bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.). Two *Bacillus thuringiensis* Berliner (Bt) cotton lines expressing either a single protein (Vip3A) or a combination of proteins (Vip3A + Cry1Ab [VipCot™]) were sampled throughout the production seasons and scored for fruiting form injury and larval survivorship. A conventional non-Bt cotton cultivar, Coker 312, was included as a negative control. Species composition and native infestation levels varied both within and across years, but Bt cotton lines had significantly fewer damaged fruiting forms and surviving larvae compared to control plants. VipCot plants had fewer damaged fruiting forms and fruiting forms infested with larvae compared to that on Vip3A plants. Seasonal patterns of efficacy generally showed VipCot plants were more durable with fewer injured fruiting forms than that recorded on Coker 312 and Vip3A, especially during peak periods of heliothine infestations. Coker 312, Vip3A, and VipCot plants artificially infested with either *H. zea* or *H. virescens* larvae were visually inspected 3 d after infestation and every 2 d thereafter until larvae were no longer detected. Fewer damaged fruiting forms and larval survivors (both species) were recorded on Vip3A and VipCot plants than on Coker 312 plants. A single *H. zea* larva injured an average of 8.6, 4.6, and 1.0 fruiting forms on

Coker 312, Vip3A, and VipCot plants, respectively. A single *H. virescens* larva injured an average of 9.2, 5.9, and 0.9 fruiting forms on Coker 312, Vip3A, and VipCot plants, respectively. The combination of two insecticidal proteins expressed in the VipCot cotton line demonstrated greater efficacy against a complex of heliothines than the single protein in the Vip3A line.

Transgenic cottons that express crystalline (Cry) proteins from the soil bacterium *Bacillus thuringiensis* Berliner (Bt) 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 been inconsistent. *H. zea* is inherently less susceptible to the Cry1Ac protein in Bollgard than *H. virescens* (Luttrell et al., 1999). Also, Cry1Ac levels expressed in plant tissue decrease as the plant ages (Adamczyk et al., 2001b; Greenplate, 1999; Olsen et al., 2005) and vegetative tissue of Bollgard plants express higher Cry protein levels compared to levels in floral structures such as pollen and flower petals (Adamczyk et al., 2001b; Gore et al., 2001; Greenplate, 1999). *H. zea* larvae are often observed feeding in flowers of Bollgard plants, which may result in relatively high levels (greater than 50%) of boll abscission (Gore et al., 2000; Smith, 1998).

The inconsistent efficacy of Bollgard against *H. zea* and occasional lepidopteran pests prompted the agrochemical industries to develop broad-spectrum transgenic insecticidal technologies. Therefore, in recent years, cotton cultivars that express two insecticidal proteins (e.g., Bollgard II® and WideStrike™) were commercialized. These traits include pyramided insecticidal Cry proteins that provide season-long, broad-spectrum control of major lepidopteran pests, which surpasses the efficacy and insect spectrum controlled with the single protein expressed in Bollgard. Several studies with Bollgard II and WideStrike have demonstrated significantly greater efficacy against *H. zea* and other lepidopteran pests

P.L. Bommireddy\*, J. Temple, and J.T. Hardke, formerly Dept. of Entomology, Louisiana State University AgCenter, 404 Life Sciences Building, Baton Rouge, LA 70803; B.R. Leonard, P. Price, and K. Emfinger, Louisiana State University Agricultural Center, Macon Ridge Research Station, 212A Macon Ridge Rd, Winnsboro, LA 71295; D. Cook, Delta Research and Extension Center, 82 Stoneville Rd., Stoneville, MS 38776

\*Corresponding author: plbommireddy@gmail.com

than Bollgard cotton (Gore et al., 2001; Leonard et al., 2005; Stewart et al., 2001; Willrich et al., 2005).

Novel transgenic cotton plants that express a vegetative insecticidal protein, Vip3A, are being developed by Syngenta Crop Protection (Lee et al., 2003). The Vip3A protein is different from Cry proteins found in all current commercial products (Bollgard, Bollgard II, and WideStrike). The Cry proteins are produced during the reproductive phase of bacteria development, enclosed in crystals, and classified as endotoxins. Vip3A, a vegetative protein, is secreted during the vegetative phase of bacterial growth and is considered an exotoxin (Micinski and Waltman, 2005; Yu et al., 1997). The initial cotton lines developed by Syngenta Crop Protection expressed Vip3A as a single protein, but the new pyramided technology, VipCot, expresses both the Vip3A and Cry1Ab proteins (McCaffery et al., 2006).

Few studies have evaluated the efficacy of VipCot technology against native and artificial infestations of heliothines or measured seasonal efficacy against these lepidopteran targets. Before 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 *H. zea* and *H. virescens*. A second objective was to estimate heliothine injury levels on 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. Cotton lines investigated in this research included the conventional non-Bt cotton cultivar, Coker 312, and Bt cotton lines expressing either a single protein (Vip3A) or a combination of proteins (Vip3A + Cry1Ab [VipCot]). The specific cotton lines for the Vip3A and VipCot plants used in this study were derived from the COT 102 event in Coker 312 germplasm. Cotton seed was planted in Gigger-Gilbert silt loam soil on 8 Jun 2005, 20 Jun 2006, and 16 Jun 2007. Plots consisted of four rows centered on 40 in and 30 ft in length. Standard cultural practices and integrated pest management strategies as recommended at the time by the Louisiana Cooperative Extension

Service were used to optimize plant development and minimize nontarget pests across the test site. The plots in these studies were not treated with any insecticides used specifically for heliothine control.

Cotton lines were evaluated by examining 25 randomly selected fruiting forms such as flower buds (squares) and bolls from the two center rows of each plot for incidence of naturally occurring heliothine damage and surviving larvae. Plots were sampled once to twice weekly from approximately 40 d after planting (DAP) to 100 DAP (mid-Jul to late-Sep). Species composition across the test areas was estimated with pheromone trap captures of heliothine adults. Wire cone traps (Hartstack et al., 1979) baited with synthetic pheromones (Hendricks et al., 1987) were used to collect *H. zea* and *H. virescens* moths. Traps were placed at several sites across the research station and collection canisters were sampled weekly. In addition, collections of larvae were examined from adjacent plots (border rows) of non-Bt cotton to 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 only those dates on which average fruiting form injury was  $\geq 5\%$  in the Coker 312 plots. Plots were sampled six, ten, and nine times during 2005, 2006, and 2007, respectively. Number of damaged fruiting forms and surviving heliothine larvae were converted to percentages, averaged across all samples, and then subjected to ANOVA using PROC MIXED (SAS Institute, 2003). To evaluate seasonal efficacy, treatments were arranged in a randomized block design with four replications. Cotton line and DAP were considered fixed effects and year and replication were included as random factors.

**Artificial Infestations of Heliothines in Field Trials.** These experiments were performed in 2006 at the Louisiana State University Agricultural Center's Macon Ridge Research Station near Winnsboro, LA (Franklin Parish). The non-Bt cotton cultivar, Coker 312, and Bt cotton lines Vip3A and VipCot were planted in Gigger-Gilbert silt loam soil on 15 Jun. Cotton lines were arranged in a randomized design across the test area. The test area was maintained with agronomic and pest management practices in a manner similar to those previously described.

The procedures for the artificial infestation study followed the general outline described by Bommireddy et al. (2007). Late-instars (L4-L5 stages) of *H. zea* and *H. virescens* were collected from sweet corn, *Zea mays*

L., and garbanzo beans, *Cicer arietinum* L., respectively, during early Jun. These collections were used to establish colonies in the laboratory and were reared for a minimum of one generation to eliminate parasitoids and pathogens and to obtain sufficient numbers at the selected age-classes of larval development. *H. zea* and *H. virescens* larvae were fed an artificial soy protein and wheat germ meridic diet (*Heliothis* premix, Ward's Natural Science, Rochester, NY) and a pinto bean-based meridic diet (Leonard et al., 1987), respectively, and reared in individual 29.5 ml plastic cups (Solo Co., Urbana, IL). Larvae were maintained at  $27 \pm 2$  °C and  $85 \pm 2\%$  relative humidity with a 14:10 light:dark photoperiod until pupation (Cook et al., 2004). Adults were held in 2.79 L cylindrical cardboard/plastic containers and fed a 10% sucrose:water solution. A single layer of cotton gauze (cheesecloth, Grade 50) was placed on top of the containers to provide a favorable surface for oviposition. Sheets of gauze containing eggs were harvested daily, placed into plastic bags, and sealed until larval eclosion. Upon eclosion, larvae were offered meridic diet until they reached the proper stage for inoculation on plants. Field infestations were completed within three to four generations of colony establishment.

Field plots of Coker 312, Vip3A, and VipCot cotton lines were thinned to three plants per meter (approximately 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 uppermost 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 and 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). Preliminary infestations with neonate heliothine larvae resulted in 100% mortality on all cotton lines including Coker 312. Therefore, a single L2 stage heliothine larva ( $72 \pm 6$  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 each of the Coker 312, Vip3A, and VipCot cotton lines were independently infested with each species on each of three separate days.

Infested plants were visually inspected 3 d after infestation (DAI) 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. Noninfested 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 that were not associated with the experiment. Treatments were arranged in a randomized block design with four replications. Number 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 repeated measures ANOVA (PROC MIXED; Littell et al., 1996). Day of evaluation is included as the repeated factor in the analysis. Effects of cotton lines and day of evaluation were considered fixed; whereas effects of year and replication were considered random factors. The mean total number of fruiting forms damaged by representative larvae for each species was subjected to ANOVA (PROC MIXED; SAS Institute, 2003).

## RESULTS

**Native Infestations of Heliothines in Field Trials.** Pheromone trap captures and samples of larvae collected from non-Bt cotton plants adjacent to test areas indicated that *H. zea* was the most common species (> 80% seasonal composition) infesting plants during all 3 y (B.R. Leonard, unpublished data). Populations of *H. virescens* were considerably lower than *H. zea* during each year. Although *H. zea* remained the dominant species (> 70%), *H. virescens* were detected and increased during the 71 to 80 DAP period. *H. virescens* did not become the dominant species ( $\approx 65\%$ ) until overall heliothine populations declined at 81 to 100 DAP.

The number of fruiting forms damaged by heliothines was significantly higher on Coker 312 compared to Vip3A and VipCot cotton lines (Table 1,  $F = 78.3$ ;  $df = 2,31$ ;  $P < 0.0001$ ). VipCot cotton also had significantly fewer heliothine-damaged fruiting forms compared to Vip3A cotton. The number of surviving larvae infesting fruiting forms was also significantly influenced by cotton type ( $F = 58.4$ ;  $df = 2,31$ ;  $P < 0.0001$ ). Larval numbers were higher on Coker 312 compared to Vip3A and VipCot cotton plants. In addition, significantly fewer heliothine larvae were recovered on VipCot plants compared to Vip3A cotton plants.

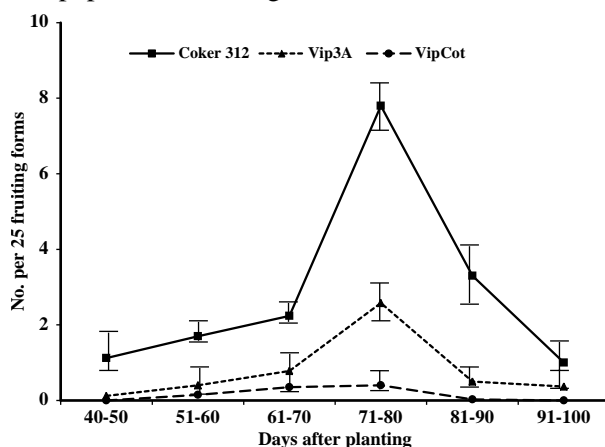
**Table 1.** Seasonal (mean  $\pm$  SE) percentage of fruiting forms damaged by heliothines (*H. zea* and *H. virescens*) and infested with larvae for non-*Bacillus thuringiensis* (Bt), Coker 312, and transgenic Bt cotton, Vip3A and VipCot, lines in Louisiana field trials from 2005 to 2007.

Cotton lines	Percent	
	Damaged forms <sup>z</sup>	Surviving larvae <sup>z</sup>
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 the same letter are not significantly different according to Fisher's protected LSD ( $\alpha = 0.05$ ).

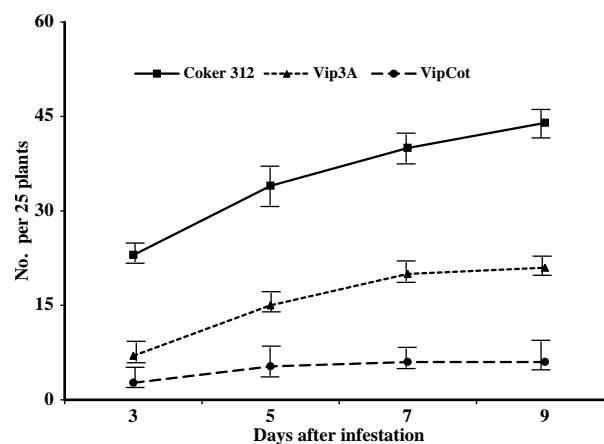
<sup>z</sup> Field trials sampled 6, 10, and 9 times during 2005, 2006, and 2007, respectively.

Heliothines damaged significantly more fruiting forms in Coker 312 plots compared to Vip3A and VipCot plots during the period of 40 to 90 DAP (Fig. 1). The number of fruiting forms damaged by heliothines was significantly influenced by cotton line ( $F = 117.0$ ;  $df = 2,54$ ;  $P < 0.0001$ ), time of evaluation ( $F = 41.4$ ;  $df = 5,54$ ;  $P < 0.0001$ ), and cotton line by time of evaluation interaction ( $F = 15.3$ ;  $df = 10,54$ ;  $P < 0.0001$ ). A single defined peak was observed in heliothine-damaged fruiting forms on Coker 312 and Vip3A plots at 71 to 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. During this time period, pheromone trap captures and samples of larvae from adjacent non-Bt plots indicated that *H. zea* remained the dominant species but low numbers of *H. virescens* were detected and populations had begun to increase.



**Figure 1.** Seasonal distribution (mean  $\pm$  SE) of *H. zea*- and *H. virescens*-damaged fruiting forms on non-*Bacillus thuringiensis* (Bt), Coker 312, and transgenic Bt cotton, Vip3A and VipCot, lines in Louisiana field trials, 2005 to 2007.

**Artificial Infestations of Heliothines in Field Trials.** Cotton line ( $F = 93.4$ ;  $df = 2,4$ ;  $P < 0.0001$ ), time of evaluation ( $F = 56.7$ ;  $df = 3,18$ ;  $P < 0.0001$ ), and cotton line by time of evaluation interaction ( $F = 11.4$ ;  $df = 6,18$ ;  $P < 0.0001$ ) were significant for *H. zea*-injured fruiting forms (Fig. 2). *H. zea* larvae injured more fruiting forms on Coker 312 compared to Vip3A and VipCot cotton plants at all rating intervals. Cumulative injury to fruiting forms also was significantly higher on Vip3A cotton compared to VipCot cotton at 3, 5, 7, and 9 DAI.



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

Cotton line ( $F = 20.7$ ;  $df = 2,4$ ;  $P < 0.0001$ ), time of evaluation ( $F = 28.2$ ;  $df = 3,18$ ;  $P < 0.0001$ ), and cotton line by time of evaluation interaction ( $F = 16.5$ ;  $df = 6,18$ ;  $P < 0.0001$ ) were significant for surviving larvae remaining on plants (Fig. 3). Significantly more *H. zea* larvae were recorded on Coker 312 plants compared with that on both Bt cotton lines at all rating intervals. At 3, 5, and 7 DAI, fewer surviving *H. zea* larvae were detected on VipCot plants compared with 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 *H. zea* larvae were found on Vip3A plants at 9 DAI, but 8.3 larvae per 25 plants were recorded on the Coker 312 cotton line.

An *H. zea* larva injured more squares ( $F = 27.8$ ;  $df = 2,4$ ;  $P < 0.01$ ), flowers ( $F = 75.5$ ;  $df = 2,4$ ;  $P < 0.01$ ), and bolls ( $F = 40.7$ ;  $df = 2,4$ ;  $P < 0.01$ ) on Coker 312 than on Vip3A and VipCot cotton lines (Fig. 4). 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 *H. zea* larva. On Vip3A plants, a single *H. zea* larva injured 4.6 fruiting forms (2.1

squares, 0.5 white flowers, and 1.9 bolls). Although VipCot plants were damaged less by *H. zea* than Vip3A plants, a low level of fruiting form injury was recorded. An *H. zea* larva damaged an average of 1.0 fruiting forms (0.6 squares, 0.2 white flowers, and 0.2 bolls) on VipCot during the 9 d evaluation period.

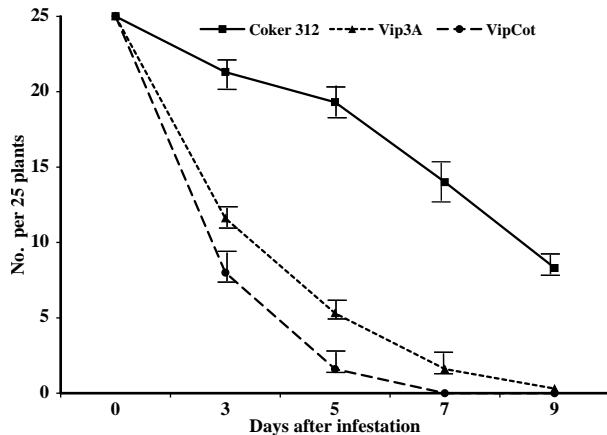


Figure 3. Surviving *H. zea* larvae (mean ± SE) recovered on non-*Bacillus thuringiensis* (Bt), Coker 312, and transgenic Bt cotton, Vip3A and VipCot, lines.

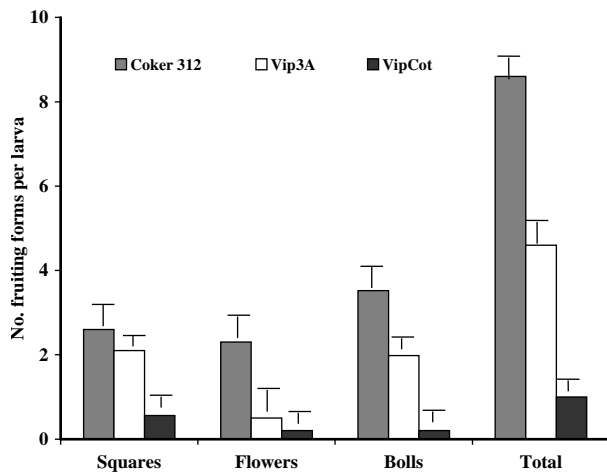


Figure 4. *H. zea* injury to fruiting forms on non-*Bacillus thuringiensis* (Bt), Coker 312, and transgenic Bt cotton, Vip3A and VipCot, lines at 9 d after inoculation in white flowers (bars represent mean ± SE of structures damaged by a single larva).

Cotton line ( $F = 77.8$ ;  $df = 2,4$ ;  $P < 0.0001$ ), time of evaluation ( $F = 66.2$ ;  $df = 3,18$ ;  $P < 0.0001$ ), and cotton line by time of evaluation interaction ( $F = 14.0$ ;  $df = 6,18$ ;  $P < 0.0001$ ) were significant for *H. virescens*-injured fruiting forms (Fig. 5). *H. virescens* larvae injured significantly more fruiting forms on Coker 312 plants compared to Vip3A and VipCot plants at all rating intervals. Cumulative injury to fruiting forms also was significantly higher on Vip3A cotton compared to VipCot cotton at 3, 5, 7, and 9 DAI.

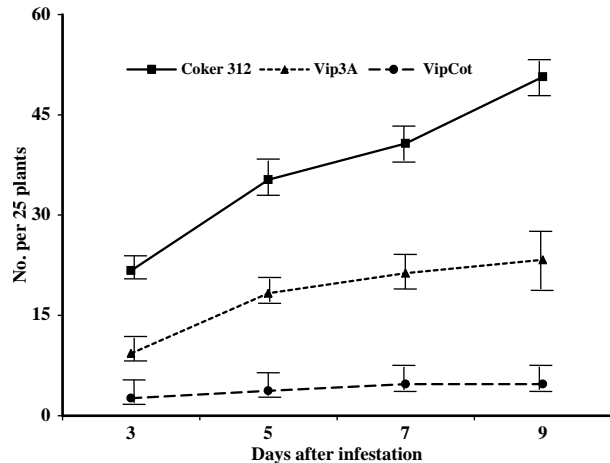


Figure 5. *H. virescens*-damaged fruiting forms (mean ± SE) on non-*Bacillus thuringiensis* (Bt), Coker 312, and transgenic Bt cotton, Vip3A and VipCot, lines.

Cotton line ( $F = 27.5$ ;  $df = 2,4$ ;  $P < 0.0001$ ), time of evaluation ( $F = 15.4$ ;  $df = 3,18$ ;  $P < 0.0001$ ), and cotton line by time of evaluation interaction ( $F = 12.2$ ;  $df = 6,18$ ;  $P < 0.0001$ ) were significant effects for surviving larvae remaining on plants (Fig. 6). Significantly more *H. virescens* larvae were recorded on Coker 312 compared with both Bt cotton lines at all rating intervals. In addition, fewer surviving *H. virescens* larvae were detected on VipCot plants compared to 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, respectively. No larvae were found on Vip3A plants by 9 DAI.

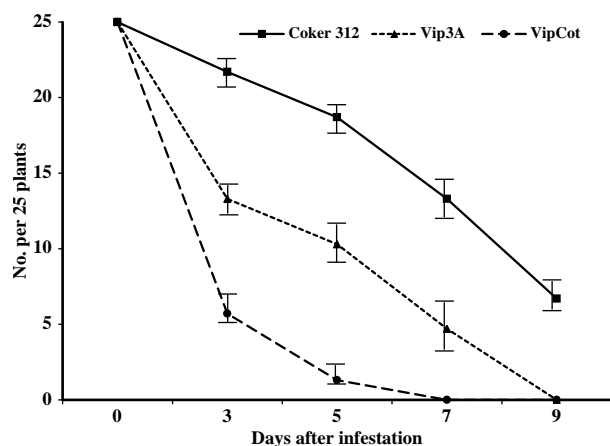
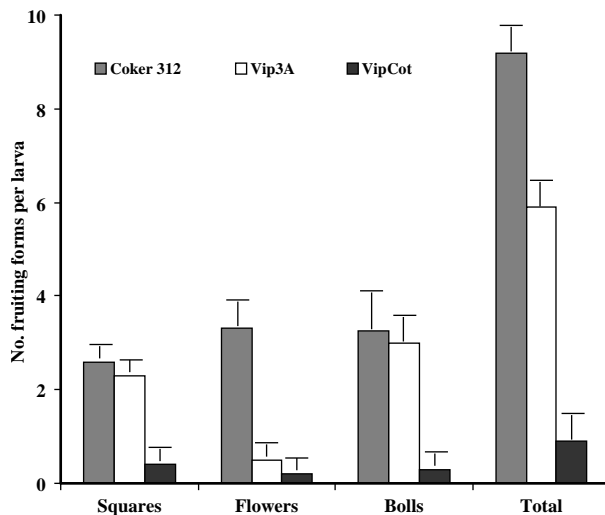


Figure 6. Surviving *H. virescens* larvae (mean ± SE) recovered on non-*Bacillus thuringiensis* (Bt), Coker 312, and transgenic Bt cotton, Vip3A and VipCot, lines.

An *H. virescens* larva injured more squares ( $F = 70.8$ ;  $df = 2,4$ ;  $P < 0.01$ ), flowers ( $F = 43.7$ ;  $df = 2,4$ ;  $P < 0.01$ ), and bolls ( $F = 37.8$ ;  $df = 2,4$ ;  $P < 0.01$ ) on Coker 312 than on Vip3A and VipCot cotton lines

(Fig. 7). 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. An *H. virescens* larva injured 0.9 fruiting forms (0.4 squares, 0.2 white flowers, and 0.3 bolls) on VipCot plants. As observed with *H. zea*, total fruiting form injury by *H. virescens* was lower on VipCot plants compared with that on Vip3A plants.



**Figure 7.** *H. virescens* injury to fruiting forms on non-*Bacillus thuringiensis* (Bt), Coker 312, and transgenic Bt cotton, Vip3A and VipCot, lines at 9 d after inoculation in white flowers (bars represent mean  $\pm$  SE of structures damaged by a single larva).

## DISCUSSION

*H. zea* and *H. virescens* larvae injured more fruiting forms on Coker 312 plants compared to that on the single protein, Vip3A, and pyramided protein, VipCot, plants during the native and artificial infestation studies. The incidence of fruiting forms infested with surviving larvae was also lower on plants of both Bt cotton lines compared with that on Coker 312 plants. In field trials evaluating the performance of single and pyramided Cry proteins expressed in cotton lines, Jackson et al. (2003) reported patterns of efficacy against *H. zea* similar to that shown 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. (2001a) 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 with 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 pests (Adamczyk et al., 2001a; Jackson et al., 2003). In the present study, the combined effects of the Cry1Ab and Vip3A proteins enhanced the overall efficacy of the VipCot line against heliothines compared to the single protein in the Vip3A line. Similar effects with these proteins have been observed in laboratory assays evaluating heliothine survivorship on cotton tissue (Bommireddy and Leonard, 2008). 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). Adamczyk and Mahaffey (2007) found *H. zea* mortality to be significantly higher on Vip3A terminal leaves compared to the non-Bt in laboratory bioassays. In the same study, no significant difference was observed in *H. virescens* mortality on Vip3A compared to non-Bt leaves.

In addition, profiling the seasonal distribution of fruiting form damage indicated that the VipCot line sustained less injury during the peak heliothine infestation period (71-80 DAP) compared with injury to Vip3A and Coker 312 fruiting forms. During that period of peak infestation, *H. zea* was the dominant species, but low levels of *H. virescens* were present. Wan et al. (2005) also documented lower *Helicoverpa armigera* (Hübner) larval densities on Bt cotton lines GK19 (Cry1Ac+ Cry1Ab) and BG1560 (Cry1Ac) throughout the season compared with that on non-Bt cotton. For many commercial Bt cotton lines expressing Cry proteins, overall levels decrease as the plant ages during the season (Greenplate, 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 Vip3A was not as consistent as that of VipCot during the season. This observation might be related to several factors such as species selectivity, infestation level, 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 damage at all rating intervals compared with that on Vip3A

and VipCot plants, regardless of heliothine species. Though injury on Vip3A cotton was significantly lower compared to Coker 312 plants, significant numbers of damaged fruiting forms were observed on Vip3A plants for both species. In addition, heliothine injury on VipCot cotton was significantly lower at all rating intervals. Studies evaluating the efficacy of Bollgard and Bollgard II against *H. zea* have shown little injury on Bollgard II compared with that on Bollgard. *H. zea* 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 DAI (Gore et al., 2003). In limited studies using artificial infestations of *H. virescens* larvae, Adamczyk and Mahaffey (2007) recovered significantly fewer larvae on Vip3A plants compared with that on non-Bt plants.

In the present study, significantly more *H. zea* and *H. virescens* larvae were recorded on Coker 312 plants compared with that on Vip3A and VipCot plants. In addition, more larvae were recorded on Vip3A plants compared with that on VipCot plants. A similar pattern of results has been observed with *H. zea* survivorship on Bollgard and Bollgard II plants. Significantly more *H. zea* larvae (2.9-fold, 6.0-fold, and 40.3-fold) were recovered on Bollgard compared with Bollgard II at 5, 7, and 9 DAI, respectively (Gore et al., 2003).

Defining the amount and type of cotton fruiting form injury produced by an individual larva is necessary information for ultimately establishing economic injury levels. The results of the present study for *H. zea* and *H. virescens* injury to fruiting forms on the non-Bt Coker 312 plants are similar with that of a number of previous studies. An individual *H. zea* and *H. virescens* can injure 6.0 to 12.1 fruiting forms on non-Bt cotton during its larval development (Anonymous, 1967; Heilman et al., 1981).

Fewer studies have examined the relationship of fruiting form injury and individual larval feeding on transgenic Bt plants. The results of the present study showed that for *H. zea* and *H. virescens*, an individual larva injured more fruiting forms on Coker 312 compared to that on Vip3A and VipCot cotton. A study by Gore et al. (2003) found that an individual *H. zea* 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. The results of these studies show that VipCot cotton lines generally sustained significantly less injury to fruiting forms and maintained lower larval infestations compared with cotton lines expressing Vip3A. In addition, the value of the pyramided Bt proteins in

VipCot can be another tool for Bt resistance management in target insect pests of cotton.

## CONCLUSIONS

These results suggest that *H. virescens* and *H. zea* were susceptible to the VipCot technology. None of the larvae for either species caused significant injury to VipCot fruiting forms compared to non-Bt fruiting forms in the field trials. In addition, no larvae were capable of completing larval development on the VipCot cotton line. In contrast, a significant level of fruiting form injury was observed on Vip3A plants. In the artificial infestations, mortality of *H. virescens* was slower on Vip3A plants compared to that of *H. zea*, which suggests differential susceptibility between species. The ability of the VipCot technology to sustain minimal injury from both heliothine species should allow this technology to become another useful tool in the cotton industry. To ensure season-long expression and efficacy against heliothine target pests, future work should focus on profiling the seasonal expression of the Vip3A and Cry1Ab protein combination on the final lines released for commercialization and among plant structures. Additional studies are needed to evaluate the efficacy of the VipCot line against a multitude of lepidopteran target pests.

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## REFERENCES

- Adamczyk, J.J., Jr., L.C. Adams, and D.D. Hardee. 2001a. Field efficacy and seasonal expression profiles for terminal leaves of single and double *Bacillus thuringiensis* toxin cotton genotypes. *J. Econ. Entomol.* 94:1589–1593.
- Adamczyk, J. J., Jr., D.D. Hardee, L.C. Adams, and D.V. Sumnerford. 2001b. Correlating differences in larval survival and development of bollworm (Lepidoptera: Noctuidae) and fall armyworm (Lepidoptera: Noctuidae) to differential expression of Cry1A(c)  $\delta$ -endotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. *J. Econ. Entomol.* 94:284–290.
- Adamczyk, J.J., and J.S. Mahaffey. 2007. Efficacy of Vip3A and Cry1Ab genotypes against various lepidopteran pests. p. 1006–1113. *In Proc. Beltwide Cotton Conf.*, New Orleans, LA. 9-12 Jan. 2007. Natl. Cotton Counc. Am., Memphis, TN.
- Anonymous. 1967. The bollworm-tobacco budworm problem in Arkansas and Louisiana. *Louis. Agric. Expt. Sta. Bull.* 720.
- Bommireddy, P.L., B.R. Leonard, and K. Emfinger. 2007. Heliothine larval behavior on transgenic cotton expressing a *Bacillus thuringiensis* insecticidal exotoxin, Vip3A. *J. Cotton Sci.* 11:177–185.
- Bommireddy, P.L., and B.R. Leonard. 2008. Survivorship of *Helicoverpa zea* and *Heliothis virescens* on cotton plant structures expressing a *Bacillus thuringiensis* vegetative insecticidal protein. *J. Econ. Entomol.* 101:1244–1252.
- Cook, D.R., B.R. Leonard, and J. Gore. 2004. Field and laboratory performance of novel insecticides against armyworms (Lepidoptera: Noctuidae). *Florida Entomol.* 87:433–439.
- Fitt, G.P., J.C. Daly, C.L. Mares, and K. Olsen. 1998. Changing efficacy of transgenic Bt cotton-patterns and consequences, p. 189–196. *In Pest Management—Future Consequences*, Vol. 1. M.P. Zalucki, R.A.I. Drew, and G.G. White (eds.). Univ. Queensland Printery, Brisbane, Australia.
- Gore, J., B.R. Leonard, G.E. Church, J.S. Russell, and T.S. Hall. 2000. Cotton boll abscission and yield losses associated with first-instar bollworm (Lepidoptera: Noctuidae) injury to nontransgenic and transgenic Bt cotton. *J. Econ. Entomol.* 93:690–696.
- Gore, J., B.R. Leonard, and J.J. Adamczyk. 2001. Bollworm (Lepidoptera: Noctuidae) survival on Bollgard and Bollgard II cotton flower buds (squares) and flowers. *J. Econ. Entomol.* 94:1445–1451.
- Gore, J., B.R. Leonard, and R.H. Gable. 2003. Distribution of bollworm, *Helicoverpa zea* (Boddie), injured reproductive structures on genetically engineered *Bacillus thuringiensis* var. *kurstaki* Berliner cotton. *J. Econ. Entomol.* 96:699–705.
- Greenplate, J.T. 1999. Quantification of *Bacillus thuringiensis* insect control protein Cry1Ac over time in Bollgard cotton fruit and terminals. *J. Econ. Entomol.* 92:1377–1383.
- Hartstack, A.W., D.E. Hendricks, J.D. Lopez, E.A. Stadelbacher, J.R. Philips, and J.A. Witz. 1979. Adult sampling, p. 105–131. *In Economic Thresholds and Sampling of Heliothis Species on Cotton, Corn, Soybeans and Other Host Plants*. South. Coop. Ser. Bull. 213. Dep. Agric. Comm., Texas A&M Univ., College Station, TX.
- Heilman, M.D., L.N. Namken, and R.H. Dilday. 1981. Tobacco budworm: Effect of early-season terminal damage on cotton yield and earliness. *J. Econ. Entomol.* 74:732–735.
- Hendricks, D.E., T.N. Shaver, and J.L. Goodenough. 1987. Development of a bioassay of molded polyvinyl chloride substrates for dispensing tobacco budworm (Lepidoptera: Noctuidae) sex pheromone bait formulations. *Environ. Entomol.* 16:605–613.
- Jackson, R.E., J.R. Bradley, Jr., and J.W. Van Duyn. 2003. Field performance of transgenic cottons expressing one or two *Bacillus thuringiensis* endotoxins against bollworm, *Helicoverpa zea* (Boddie). *J. Cotton Sci.* 7:57–64.
- 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. *Appl. Environ. Microbiol.* 69:4648–4657.
- Leonard, B.R., J.B. Graves, T.C. Sparks, and A.M. Pavloff. 1987. Susceptibility of bollworm and tobacco budworm larvae to pyrethroid and organophosphate insecticides. p. 320–324. *In Proc. Beltwide Cotton Conf.*, Dallas, TX. 5-8 Jan. 1987. Natl. Cotton Counc. Am., Memphis, TN.
- Leonard, B.R., R. Gable, K. Emfinger, K. Tindall, J. Temple, and L. Bommireddy. 2005. Louisiana research efforts with WideStrike and VipCot pest management technologies. p. 1433–1436. *In Proc. Beltwide Cotton Conf.*, New Orleans, LA. 4-7 Jan. 2005. Natl. Cotton Counc. Am., Memphis, TN.
- Littell, R.C., G.A. Milliken, W.W. Stroup, and R.D. Wolfinger. 1996. SAS<sup>®</sup> system for mixed models. SAS Institute, Cary, NC.
- Luttrell, R.G., L. Wan, and K. Knighten. 1999. Variation in susceptibility of Noctuid (Lepidoptera) larvae attacking cotton and soybean to purified endotoxin proteins and commercial formulations of *Bacillus thuringiensis*. *J. Econ. Entomol.* 92:21–32.
- McCaffery, A., M. Caprio, R. Jackson, M. Marcus, T. Martin, D. Dickerson, D. Negrotto, D. O'Reilly, E. Chen, and M. Lee. 2006. Effective IRM with a novel insecticidal protein, Vip3A. p. 1229–1235. *In Proc. Beltwide Cotton Conf.*, San Antonio, TX. 3-6 Jan. 2006. Natl. Cotton Counc. Am., Memphis, TN.



- Micinski, S., and B. Waltman. 2005. Efficacy of VipCot for control of the bollworm/tobacco budworm complex in Northwest Louisiana. p. 1239–1242. *In Proc. Beltwide Cotton Conf.*, New Orleans, LA. 4-7 Jan. 2005. Natl. Cotton Counc. Am., Memphis, TN.
- Olsen, K.M., J.C. Daly, H.E. Holt, and E.J. Finnegan. 2005. Season-long variation in expression of Cry1Ac gene and efficacy of *Bacillus thuringiensis* toxin in transgenic cotton against *Helicoverpa armigera* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 98:1007–1017.
- Parker, R.D., and S.D. Livingston. 2005. Results of field experiments with transgenic cotton varieties (VipCot and WideStrike) in the Texas coastal blend. p. 1687–1693. *In Proc. Beltwide Cotton Conf.*, New Orleans, LA. 4-7 Jan. 2005. Natl. Cotton Counc. Am. Memphis, TN.
- SAS Institute. 2003. SAS version 9.1 for Windows. SAS Institute, Cary, NC.
- Smith, R.H. 1998. Year two of Bollgard behind boll weevil eradication: Alabama observations. p. 965–966. *In Proc. Beltwide Cotton Conf.*, San Diego, CA. 5-9 Jan. 1998. Natl. Cotton Counc. Am., Memphis, TN.
- Stewart, S.D., J.J. Adamczyk, Jr., K.S. Knighten, and F.M. Davis. 2001. Impact of Bt cottons expressing one or two insecticidal proteins of *Bacillus thuringiensis* (Berliner) on growth and survival of noctuid (Lepidoptera) larvae. *J. Econ. Entomol.* 94:752–760.
- Wan, P., Y. Zhang, K. Wu, and M. Huang. 2005. Seasonal expression profiles of insecticidal protein and control efficacy against *Helicoverpa armigera* for Bt cotton in the Yangtze river valley of China. *J. Econ. Entomol.* 98:195–201.
- Willrich, M.M., L. B. Braxton, J.S. Richburg, R.B. Lassiter, V.B. Langston, R.A. Haygood, J.M. Richardson, F.J. Haile, R.M. Huckaba, J.W. Pellow, G.D. Thompson, and J.P. Mueller. 2005. Field and laboratory performance of WideStrike insect protection against secondary lepidopteran pests. p. 1262–1268. *In Proc. Beltwide Cotton Conf.*, New Orleans, LA. 4-7 Jan. 2005, Natl. Cotton Counc. Am., Memphis, TN.
- Yu, C.G., M.A. Mullins, G.W. Warren, M. Koziel, and J.K.J. Estruch. 1997. The *Bacillus thuringiensis* vegetative insecticidal protein, Vip3A, lyses midgut epithelium cells of susceptible insects. *Appl. Environ. Microbiol.* 63:532–536.