

ARTHROPOD MANAGEMENT AND APPLIED ECOLOGY

Heliothine Larval Behavior on Transgenic Cotton Expressing a *Bacillus thuringiensis* Insecticidal Exotoxin, Vip3A

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ABSTRACT

Field studies were performed during 2005 and 2006 to determine bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), larval behavior on conventional non-transgenic cotton plants (Coker 312), and on transgenic *Bacillus thuringiensis* Berliner (Bt) plants expressing the Vip3A protein or a combination of Vip3A + Cry 1Ab proteins (VipCot). Plants representing each of the three cotton lines were infested with a single, 2-d-old bollworm or tobacco budworm larva during pre-flowering and flowering stages of development. On pre-flowering cotton plants, significantly more bollworm and tobacco budworm larvae migrated from the site of infestation (terminal region of the plant) on Bt (Vip3A and VipCot) cotton plants than larvae placed on non-Bt Coker 312 cotton plants. Higher numbers of larvae for both species were recovered on traps beneath Bt cotton plants than on traps beneath non-Bt cotton plants. During the flowering stages of cotton plant development, similar numbers of both species were recovered from terminals of the non-Bt, Vip3A, and VipCot plants at 1 h and 3 h after infestation. Significantly more bollworm and tobacco budworm larvae were observed on flower buds (squares) of non-Bt cotton than on squares of Vip3A and VipCot at all evaluation intervals. Within 24 h, bollworm larvae moved 1.5, 2.8, and 0.8 main stem nodes below the terminal on Vip3A, VipCot, and Coker 312 cottons, respectively. At the same time interval, tobacco budworm larvae moved 2.0, 2.8, and 0.9 main stem nodes below the terminal on Vip3A, VipCot, and Coker 312 cottons, respectively.

During the study, no significant differences in bollworm and tobacco budworm larval behavior were detected between the Vip3A and VipCot cotton lines. The results of this study are similar to those of previous studies that have recorded larval movement on Bt cotton plants expressing single or multiple cry proteins. It is likely that the current sampling protocols used to evaluate performance of commercial Bt cotton plants and supplemental IPM strategies for bollworm and tobacco budworm also can be eventually used for VipCot cultivars.

Transgenic cotton cultivars that express δ -endotoxin from the bacterium, *Bacillus thuringiensis* Berliner (Bt), have been widely adopted as alternative IPM strategies to conventional foliar sprays for management of specific lepidopteran pests. Two of the primary targets of the Bt cotton technologies in the United States are commonly referred to as heliothines and include the bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.). These species have historically been significant economic pests of cotton across the U.S. Cotton Belt, because of the cost of control strategies or associated yield losses (Williams, 2006).

All commercial Bt cotton cultivars provide excellent control of tobacco budworm, but supplemental foliar applications of insecticides are often necessary to manage bollworm (Leonard et al., 2001). In cage studies, more than 2% of tobacco budworm larvae survived on transgenic cottons expressing a single crystal (Cry) insecticidal protein endotoxin (Benedict et al., 1993). The results of field studies showed that survival of tobacco budworm ranged from 0 to 8% on transgenic Bt plants, while larval survival ranged from 49 to 88% on non-transgenic plants (Jenkins et al., 1993). On commercial Bollgard plants expressing only the Cry1Ac protein, high bollworm populations produced 14% boll injury (Mahaffey et al., 1995). Bollworm larvae are often observed feeding in flowers of Bollgard plants and can result in relatively high levels (more than 50%) of boll abscission (Smith, 1998; Gore et al., 2000).

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Expression levels and distribution of Cry1Ac protein are influenced by plant age, as well as location of vegetative and fruiting structures on plants. Relative expression levels of this protein decrease as the cotton plant ages during the season (Greenplate, 1999). In addition, not all plant structures (i.e. leaves, squares, flowers, and bolls) express similar concentrations of Cry1Ac protein (Adamczyk et al., 2001; Gore et al., 2001; Oslen et al., 2005); therefore, the behavior and distribution of tobacco budworm and bollworm larvae on a Bt cotton plant may be influenced by this intra-plant variation in protein expression. Tobacco budworm larvae placed on Bt plants abandon those plants more often than larvae on non-Bt cotton plants (Benedict et al., 1992; 1993; Parker and Luttrell, 1999). A reduction in feeding activity by bollworm larvae compared with larvae offered the same diet without Bt proteins was observed in two studies using meridic diets containing purified Bt proteins (Greenplate et al., 1998; Akin et al., 2001). On intact cotton plants, bollworm larvae migrated from terminal leaves treated with a foliar Bt spray to adjacent expanded leaves or completely away from the plant terminal region (Jyoti et al., 1996). In another study, bollworm larvae placed on Bollgard plants migrated from the site of infestation more often and more rapidly than larvae on non-Bollgard plants (Gore et al., 2002).

Transgenic Bt technologies have advanced during recent years, and cotton cultivars that express two insecticidal proteins (Bollgard II and WideStrike) have improved control of bollworm and other lepidopteran pests greater than that provided by the single protein expressed in Bollgard (Stewart et al., 2001; Willrich et al., 2005). Scientists at Syngenta Crop Protection (Greensboro, NC) have also used genetic engineering protocols to develop novel transgenic cotton technology that expresses the Vip (vegetative insecticidal protein) 3A from Bt (Lee et al., 2003). Their initial cotton lines only expressed Vip3A as a single protein, but the new VipCot plants express both Vip3A and Cry1Ab proteins (McCaffery et al., 2006). The Vip3A protein has demonstrated significant levels of toxicity to lepidopteran targets, but also has exhibited considerable selectivity to non-target invertebrates (Mascarenhas, 2004; Micinski and Waltman, 2005; Whitehouse et al., 2007).

The Vip3A protein is different from the Cry proteins expressed in Bollgard, Bollgard II, and WideStrike cotton cultivars. It is secreted during the vegetative phase of bacterial development, whereas

the insecticidal Cry proteins are produced during the reproductive phase of bacterial development, enclosed in crystals, and classified as endotoxins (Micinski and Waltman, 2005; Yu et al., 1997). These differences between cotton plants expressing the Vip3A protein and those cotton plants expressing Cry proteins could provide a basis for reducing the potential of insect cross-resistance (McCaffery et al., 2005).

No research has examined tobacco budworm or bollworm larval behavior on transgenic cotton plants expressing Vip3A or VipCot proteins. The objective of this study was to observe and record the behavior of both pests on cotton plants expressing these proteins, which is necessary to validate or refine the current IPM strategies for transgenic Bt cotton.

MATERIALS AND METHODS

This study was performed at the Louisiana State University Agricultural Center Macon Ridge Research Station near Winnsboro, LA, (Franklin Parish) during 2005 and 2006. The conventional non-Bt cotton cultivar, Coker 312, and Bt cotton lines expressing either a single protein (Vip3A) or combination of proteins (Vip3A + Cry 1Ab [VipCot]) were planted in a Gigger-Gilbert silt loam soil every 3 wk from 9 June to 10 July during both years. This temporal planting pattern provided a wide range of plant maturities at the appropriate stages for infestations. 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).

Insects. Bollworm and tobacco budworm larvae were collected from sweet corn (*Zea mays* L.) and garbanzo beans (*Cicer arietinum* L.) during early June of each year. Colonies from those collections were established in the laboratory and reared for a minimum of one generation to eliminate parasitoids and pathogens and to obtain sufficient numbers at the proper stages of larval development. Bollworm larvae were fed an artificial soy protein, wheat germ based diet (*Heliothis* premix; Stonefly Industries Inc.; Bryan, TX). Tobacco budworm larvae were fed a pinto bean based diet (Leonard et al., 1987) in individual 29.5-ml plastic cups (Solo Co.; Urbana, IL). Heliiothine larvae were maintained at 27 ± 2 °C and $85 \pm 2\%$ relative humidity with a 14:10 light:dark photoperiod until pupation. Adults of both species were held in 2.79-L cylindrical cardboard/plastic

containers and fed a 10% sucrose solution. A single layer of cheesecloth was placed on top of the containers to provide an adequate surface for oviposition. The oviposition sheets were harvested daily and placed into plastic bags until larval eclosion. Upon eclosion, larvae were offered the meridic diet for approximately 48 h.

Infestation of larvae on pre-flowering cotton plants. Seedlings in the Coker 312, Vip3A, and VipCot plots were thinned to 3 plants per meter (one plant per row-foot) before infestation to prevent interplant movement of larvae. Those plants designated for infestation were examined for the presence of eggs and larvae. Only those plants without a natural heliothine infestation were used in these studies. A trap (40.6 × 40.6-cm sheet of cardstock) coated with Tanglefoot (Tanglefoot Company; Grand Rapids, MI) was placed on the soil beneath each plant prior to infestation. This trap placement was designed to capture any larva that exhibited “spin-down” behavior from the site of infestation and to demonstrate larval avoidance of the Bt toxin(s). A single first-instar (48 ± 6 h old) heliothine larva was placed in the terminal region of each plant using a small camel’s hair brush. The infested plants were rated at 1, 3, 6, and 24 h after infestation by whole-plant inspection. The number of nodes that a larva migrated from the original infestation site and incidence of avoidance (larval collection on the sticky trap) were recorded for each infested plant. This study consisted of 10 replications during the 2 yr (2005 and 2006) period. The cotton lines were arranged in a completely randomized design across the test area. Replications were represented by the day of infestation. Twenty plants of the Coker 312, Vip3A, and VipCot cotton lines were infested on each day. A total of 200 plants were infested during both years. All data were converted to percentages based on the number of plants infested on a given day and analyzed using repeated measures analysis of variance (PROC MIXED; ver. 9.1; SAS Institute; Cary, NC). Data were analyzed separately for larval species.

Infestation of larvae on flowering cotton plants. Cotton plants representing the Coker 312, Vip3A, and VipCot cotton lines during flowering stages (8-9 main stem nodes above a first position sympodial white flower extending to the plant terminal [NAWF]) were infested with first instar (48 ± 6 h old) bollworm or tobacco budworm larvae. The procedures and experimental design for larval infestations during the flowering stages of development

were similar to those described for pre-flowering cotton except the sticky trap used to measure larval avoidance was not used. Plants were rated at 1, 3, 6, and 24 h after infestation by visually examining infested plants. The number of larvae that migrated from the original infestation site (plant terminal) and plant structure (terminal, square, flower, or boll) infested with larvae were recorded. Data were analyzed using repeated measures analysis of variance (PROC MIXED; ver. 9.1; SAS Institute; Cary, NC).

RESULTS

Heliothine larval movement on pre-flowering cotton plants. Bollworm larval behavior was significantly different among the three cotton lines. Cotton line ($F = 35.19$; $df = 1, 12$; $P = 0.001$), time of evaluation ($F = 42.20$; $df = 3, 36$; $P = 0.001$), and the cotton line by time of evaluation interaction ($F = 3.38$; $df = 6, 36$; $P = 0.01$) effects were significant for percentages of larval-infested plant terminals (Fig. 1). The percentages of plant terminals that contained bollworm larvae on the Coker 312 plants (77.3 to 97.7%) were significantly higher than on the Vip3A (32.8 to 75.0%) and VipCot (30.9 to 85.0%) cotton plants at all rating intervals. Bollworm larvae migrated from the plant terminals of all three cotton lines, but most of the larval movement in the Coker 312 line had occurred within 3 h after infestation. On Vip3A and VipCot plants, larval movement from the site of infestation did not cease declining until 6 h after infestation.

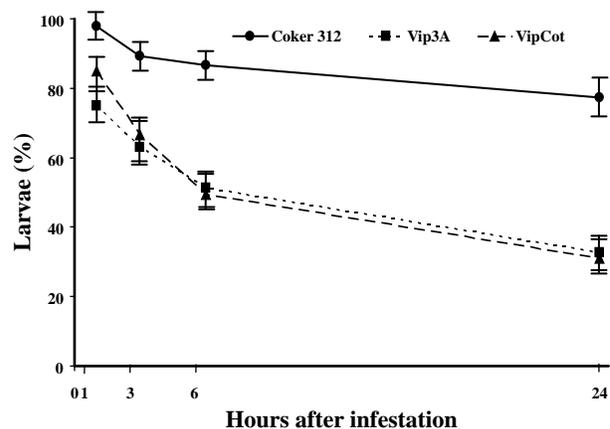


Figure 1. Percentage (\pm SE) of non-Bt and Bt cotton plant terminals infested with bollworm larvae.

Cotton line ($F = 25.19$; $df = 1, 12$; $P = 0.001$), time of evaluation ($F = 14.65$; $df = 3, 12$; $P = 0.0003$), and the cotton line by time of evaluation interaction ($F = 6.90$; $df = 6, 36$; $P = 0.01$) was significant for percentages of bollworm larvae recovered from

sticky traps beneath pre-flowering cotton plants (Fig. 2). Higher percentages of bollworm larvae were recovered on traps beneath Vip3A (24.3 to 55.3%) and VipCot (15.0 to 58.3%) plants than on traps below the Coker 312 (2.8 to 17.7%) plants at all rating intervals ($P = 0.001$). There was no significant difference in the percentage of larvae collected on traps below the Vip3A and VipCot plants during any evaluation period.

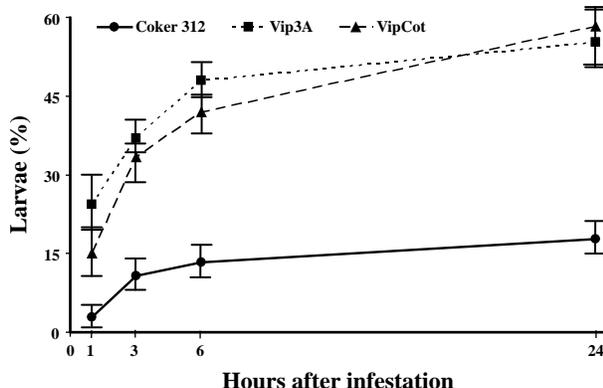


Figure 2. Percentage (\pm SE) of bollworm larvae recovered from sticky traps beneath non-Bt and Bt cotton plants.

Intra-plant vertical migration of bollworm larvae was influenced by cotton line ($F = 4.54$; $df = 2, 7$; $P = 0.01$) and time of evaluation ($F = 13.92$; $df = 3, 21$; $P < 0.0001$). Larvae migrated significantly farther from the infestation site on the Vip3A and VipCot plants than on the Coker 312 plants at 3, 6, and 24 h after infestation (Fig. 3). Within 24 h, larvae were recorded on sympodia at 0.8, 1.5, and 2.8 main stem nodes below the terminal on the Coker 312, Vip3A, and VipCot plants, respectively. In addition, larval migration increased from the time of infestation to the endpoint (24 h after infestation) of the experiment. There was a significant interaction ($F = 4.49$; $df = 6, 21$; $P < 0.004$) between cotton line and time of evaluation for bollworm larval movement. Larvae dispersed farther on VipCot plants compared with those on the Vip3A plants at 6 and 24 h after infestation.

Tobacco budworm larval behavior also was significantly influenced by the Vip3A and VipCot cotton lines. Cotton line ($F = 29.49$; $df = 2, 17$; $P < 0.0001$), time of evaluation ($F = 42.76$; $df = 3, 51$; $P < 0.0001$), and the cotton line by time of evaluation interaction ($F = 12.31$; $df = 6, 51$; $P < 0.0001$) affected the percentages of larval-infested plant terminals (Fig. 4). Tobacco budworm larvae were more common in the plant terminals of the Coker

312 plants than in the plant terminals of Vip3A and VipCot cotton plants at 3, 6, and 24 h after infestation. The percentages of larval-infested plant terminals for all three cotton lines declined across the entire evaluation period. By 24 h after infestation, percentages of Coker 312, Vip3A, and VipCot plant terminals that contained larvae were 72.4%, 41.0%, and 38.6%, respectively. Similar frequencies of larvae were observed in Vip3A and VipCot terminals within each evaluation interval.

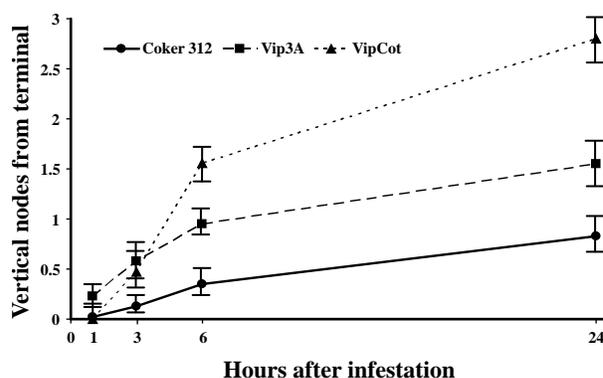


Figure 3. Distribution (\pm SE) of bollworm larvae recorded on main stem node sympodia below plant terminals on non-Bt and Bt cotton plants.

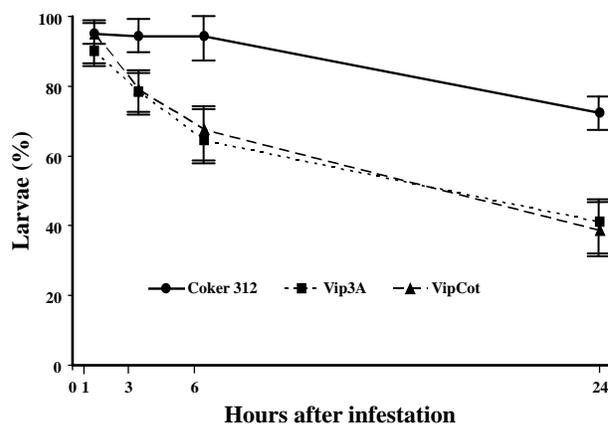


Figure 4. Percentage (\pm SE) of non-Bt and Bt cotton plant terminals infested with tobacco budworm larvae.

The percentages of tobacco budworm larvae collected on sticky traps placed beneath the plants were significantly affected by cotton line ($F = 22.19$; $df = 2, 17$; $P < 0.0001$), time of evaluation ($F = 46.30$; $df = 3, 51$; $P < 0.0001$), and the cotton line by time of evaluation interaction ($F = 8.13$; $df = 6, 51$; $P < 0.0001$). The percentage of larvae found on traps beneath Vip3A and VipCot plants was significantly higher than on traps beneath Coker 312 plants at 3, 6, and 24 h after infestation

(Fig. 5). The percentage of tobacco budworm larvae observed on traps beneath Coker 312, Vip3A, and VipCot cotton plants across all rating intervals ranged from 5.0 to 11.4%, 10.0 to 41.0%, and 5.0 to 50.0%, respectively. Vip3A and VipCot cotton lines were not different in the percentage of larvae recovered from traps at any evaluation interval.

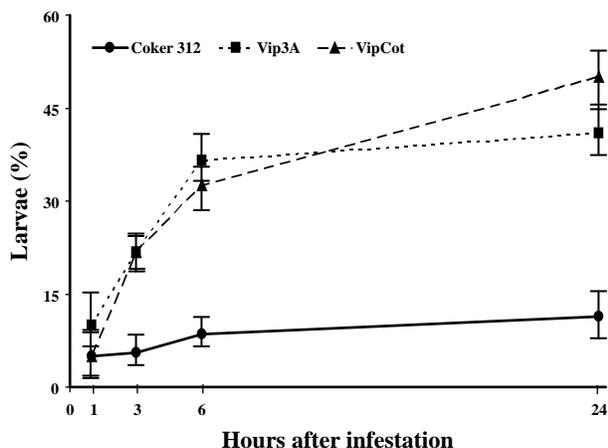


Figure 5. Percentage (\pm SE) of tobacco budworm larvae recovered from sticky traps beneath non-Bt and Bt cotton plants.

Tobacco budworm larval migration down the main stem was influenced by cotton line ($F = 24.66$; $df = 2, 9$; $P = 0.0002$), time of evaluation ($F = 59.71$; $df = 3, 27$; $P < 0.0001$), and by the cotton line and time of evaluation interaction ($F = 11.86$; $df = 6, 27$; $P < 0.0001$). Larvae migrated significantly farther from the infestation site on the Vip3A and VipCot plants than larvae on the Coker 312 plants at 3, 6, and 24 h after infestation (Fig. 6). Larvae were found on sympodia at an average of 0.9, 2.0, and 2.8 main stem nodes below the terminal at 24 h after infestation on the Coker 312, Vip3A, and VipCot plants, respectively. Tobacco budworm larvae migrated significantly farther on VipCot plants than on Vip3A plants by 24 h after infestation.

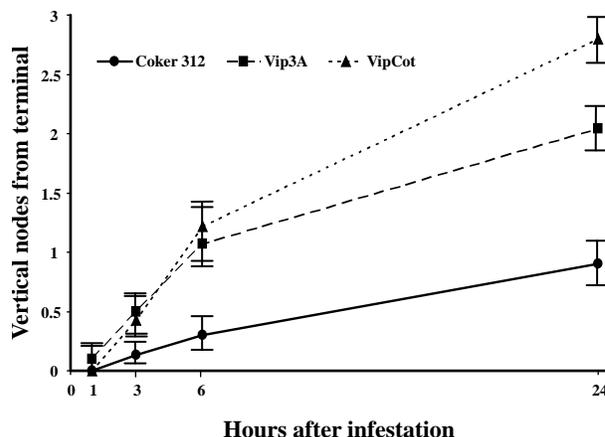


Figure 6. Distribution (\pm SE) of tobacco budworm larvae recorded on main stem sympodia below plant terminals on non-Bt and Bt cotton plants.

Heliothine larval movement on flowering cotton plants. Intra-plant movement and preferred feeding sites for bollworm larvae were significantly different among the non-Bt Coker 312, Vip3A, and VipCot plants (Table 1). Cotton line ($F = 48.64$; $df = 2, 9$; $P < 0.001$), time of evaluation ($F = 14.40$; $df = 3, 27$; $P < 0.001$), and the cotton line by time of evaluation interaction ($F = 24.58$; $df = 6, 27$; $P < 0.001$) effects were significant for numbers of plant terminals infested with bollworm larvae. Higher numbers of larvae were recorded on Coker 312 plant terminals compared with numbers on the Vip3A and VipCot cotton lines at 6 and 24 h after infestation. Numbers of larvae in Coker 312 plant terminals decreased by two-fold at 24 h after infestation. A similar decrease was observed by 6 h after infestation on the Vip3A and VipCot plants.

Cotton line ($F = 11.46$; $df = 2, 9$; $P < 0.001$), time of evaluation ($F = 19.99$; $df = 3, 27$; $P < 0.001$), and the cotton line by time of evaluation interaction ($F = 13.10$; $df = 6, 27$; $P < 0.001$) also had significant effects on bollworms in cotton squares (Table 1). Numbers of larvae on Coker 312 squares were

Table 1. Number (Mean \pm SE) of bollworms observed on non-Bt and Bt plant structures during flowering at selected time intervals after infestation of 2-d-old larvae in the plant terminal

Line	Plant terminal (h after infestation) ^z				Square (h after infestation) ^z			
	1	3	6	24	1	3	6	24h
Coker 312	9.75 \pm 0.41a	8.12 \pm 0.25a	7.75 \pm 0.25a	3.75 \pm 0.32a	4.87 \pm 0.25a	8.00 \pm 0.18a	8.12 \pm 0.23a	9.25 \pm 0.47a
Vip3A	9.12 \pm 0.29a	8.25 \pm 0.24a	3.25 \pm 0.09b	2.62 \pm 0.25b	2.50 \pm 0.27b	3.00 \pm 0.41b	2.75 \pm 0.28b	2.62 \pm 0.16b
VipCot	9.18 \pm 0.25a	8.44 \pm 0.30a	3.18 \pm 0.20b	2.69 \pm 0.25b	2.25 \pm 0.25b	2.75 \pm 0.19b	2.75 \pm 0.24b	2.50 \pm 0.29b

^z Means within a column followed by same letter are not significantly different according to Tukey's Studentized Range Test ($P = 0.05$).

significantly higher compared with number of larvae on Vip3A and VipCot cotton squares at all rating intervals. At 1 h after infestation numbers of bollworm larvae were approximately two-fold greater on Coker 312 squares compared with numbers on Vip3A and VipCot squares. There were no differences in numbers of bollworms on Vip3A and VipCot cotton squares. Bollworm larvae found on Coker 312 squares increased about two-fold within 24 h after infestation; however, there was no significant change in the numbers of bollworms on Vip3A and VipCot squares across the entire sampling period.

The results for tobacco budworm larval movement recorded on Coker 312, Vip3A, and VipCot plants were similar to those for bollworm larvae (Table 2). Cotton line ($F = 19.31$; $df = 2, 9$; $P = 0.006$), time of evaluation ($F = 31.08$; $df = 3, 27$; $P < 0.001$), and the cotton line by time of evaluation interaction ($F = 20.60$; $df = 6, 27$; $P < 0.001$) effects were significant for numbers of plant terminals infested with tobacco budworm. Fewer tobacco budworm larvae remained in terminals of Vip3A and VipCot plants than on Coker 312 plants at 6 and 24 h after infestation. Similar numbers of larvae were recorded on Vip3A and VipCot cotton terminals at all sampling intervals. Numbers of tobacco budworm larvae in Coker 312, Vip3A, and VipCot cotton terminals decreased with each successive rating interval.

Cotton line ($F = 20.33$; $df = 2, 9$; $P < 0.0001$), time of evaluation ($F = 30.56$; $df = 3, 27$; $P < 0.0001$), and the cotton line by time of evaluation interaction ($F = 13.22$; $df = 6, 27$; $P < 0.0001$) also had significant effects on tobacco budworms in squares (Table 2). Numbers of larvae were significantly higher on Coker 312 squares compared with those on Vip3A and VipCot squares at all rating intervals. At 1 h after infestation, numbers of tobacco budworm larvae were more than two-fold higher on Coker 312 squares compared with that on

Vip3A and VipCot squares. There were no differences in numbers of tobacco budworms on Vip3A and VipCot squares. Similar to the observation for bollworms, tobacco budworm larvae found on Coker 312 squares increased greater than 1.8-fold by 24 h after infestation, but no differences in larvae on Vip3A and VipCot squares were recorded across the entire sampling period.

DISCUSSION

Bollworm and tobacco budworm larval movement from cotton plant terminals was significantly influenced by the Bt cotton lines evaluated in the present study. Both heliothine species dispersed more rapidly on the two Bt lines than on non-Bt (Coker 312) plants. In addition, by 24 h after infestation, bollworm and tobacco budworm larvae were detected farther down the plant on Vip3A (0.5 to 1.0 nodes) and VipCot (1.4 to 1.75 nodes) plants compared with Coker 312 plants. Larval dispersal was generally similar between Vip3A and VipCot lines with the exception of larval distribution on sympodia of flowering plants at 6 and 24 h after infestation. Gore et al. (2002) reported that bollworm larvae moved approximately 3.3 nodes farther down on Bollgard cotton compared with larvae on a non-Bollgard line at 24 h after infestation. In this study, significantly more larvae were observed on traps beneath Vip3A and VipCot plants compared with those on traps beneath Coker 312 plants. Gore et al. (2002) recovered approximately three-fold more bollworm larvae on traps beneath Bollgard cotton plants compared with traps beneath non-Bollgard cotton plants. Tobacco budworm larvae exhibited similar behavior on Bt plants and spent five-fold more time in spin-down behavior on Bt plants than on non-Bt cotton plants (Benedict et al., 1992). In a similar study, more tobacco budworms (13%)

Table 2. Number (Mean \pm SE) of tobacco budworms observed on non-Bt and Bt plant structures during flowering at selected time intervals after infestation of 2-d-old larvae in the plant terminal

Line	Terminal (h after infestation) ^z				Square (h after infestation) ^z			
	1	3	6	24	1	3	6	24
Coker 312	9.50 \pm 0.31a	7.75 \pm 0.20a	7.25 \pm 0.25a	3.85 \pm 0.18a	5.00 \pm 0.41a	7.50 \pm 0.29a	8.25 \pm 0.25a	9.25 \pm 0.25a
Vip3A	9.25 \pm 0.23a	8.75 \pm 0.28a	3.00 \pm 0.17b	2.62 \pm 0.25b	2.00 \pm 0.09b	2.50 \pm 0.25b	3.00 \pm 0.11b	2.50 \pm 0.39b
VipCot	9.25 \pm 0.25a	8.50 \pm 0.16a	3.50 \pm 0.28b	3.00 \pm 0.45b	2.25 \pm 0.24b	2.75 \pm 0.23b	2.50 \pm 0.43b	2.50 \pm 0.08b

^zMeans within a column followed by same letter are not significantly different according to Tukey's Studentized Range Test ($P = 0.05$).

infested on Bollgard plants migrated to adjacent plants than larvae infested on non-Bollgard plants (Parker and Luttrell, 1999). Benedict et al. (1992, 1993) observed that higher numbers of tobacco budworm migrated from terminals of Bt cotton than on non-Bt cotton.

On flowering stage cotton plants, bollworm and tobacco budworm were observed on approximately 35 to 40% of the terminals on Coker 312, Vip3A, and VipCot cotton plants at 3 h after infestation. On Vip3A and VipCot plants, few larvae were observed in terminals than on Coker 312 plants at 6 and 24 h after infestation. Heliothine larvae either began to disperse rapidly after infestation or attempted to avoid feeding on the Vip3A or VipCot plants by exhibiting spin-down behavior. In addition, heliothine larval numbers were significantly higher on Coker 312 squares than on Vip3A and VipCot squares at all rating intervals. Heliothine larvae were not observed in flowers and bolls on infested plants for any of the cotton lines in this study. On the Vip3A and VipCot plants many of those insects likely either left the plant or were killed by the Bt protein(s). On Coker 312 plants, the insects appeared to remain on squares or in the terminal. Gore et al. (2002) observed more larvae on non-Bollgard squares at 24 h after infestation, which is consistent with the results of the present study. Pietrantonio and Heinz (1999) also observed more heliothine larvae in the top 20 nodes of non-Bt cotton plants than on Bt cotton plants.

These differences in the intra-plant migration patterns and distribution of heliothine larvae are likely related to the avoidance behavior caused by Bt protein(s) present in the transgenic cotton plants. After 24 h, 88% of bollworm larvae were found in cups containing non-Bt leaf tissue, whereas only 68% and 53% of larvae were observed in cups containing single and dual toxin plant tissue, respectively (Akin et al., 2001). Prior to the development of cottons which were transformed to express Bt protein, Jyoti et al. (1996) showed bollworm larvae dispersed from plant terminals to nearby expanded leaves within 6 h of a Bt spray application to those cotton terminals. Heliothine detection of Bt insecticidal proteins appears to occur for both foliar spray residues on leaf surfaces or expression throughout leaf tissue. The avoidance behavior is probably a survival mechanism that forces the insects to migrate more rapidly and farther to locate suitable non-toxic plant structures.

CONCLUSIONS

The results of the present study with Vip3A and VipCot are similar to the previous reports of Bt Cry proteins in cotton plants and their significant effects on heliothine behavior. Currently, there are no published reports documenting heliothine larval behavior on transgenic cotton expressing the vegetative insecticidal protein, Vip3A. As a result of the behavioral effects of Bt on heliothines, bollworm larvae have been commonly found feeding in white flowers in Bollgard cotton fields. The current scouting protocols appropriate for sampling commercial Bt cultivars will likely be sufficient for cotton plants expressing the VipCot technologies. Several of the cooperative extension services in cotton production states recommend sampling fruiting structures, such as flowers and bolls, to detect bollworm infestations in commercial Bt cotton fields. Scouting for heliothines in commercial cotton fields expressing the Vip3A and VipCot traits should include an examination of vegetative and reproductive structures below the plant terminal. The heliothine complex of bollworm and tobacco budworm are primary targets of Bt traits in cotton, and satisfactory control is usually obtained without foliar oversprays. In instances where additional control is warranted, accurately detecting heliothine larval distribution in Bt cotton plants is necessary to provide the information for appropriate management decisions.

ACKNOWLEDGEMENT

We wish to thank the numerous student workers at the Macon Ridge Research Station for their assistance in this study. The financial support provided by the Louisiana State University Agricultural Center, Cotton Incorporated, and Louisiana's cotton producers to complete this project is also appreciated. This manuscript is published with the approval of the Director of the Louisiana Agricultural Experiment Station as manuscript number 07-26-0342.

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