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EFFICACY OF VIP3A AND CRY1AB TRANSGENIC TRAITS IN COTTON AGAINST VARIOUS LEPIDOPTERAN PESTS

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ABSTRACT

From 2004 through 2005, plots of experimental transgenic cotton lines containing the vegetative insecticidal protein, Vip3A; δ-endotoxin, Cry1Ab; and both Vip3A and Cry1Ab were evaluated for efficacy against certain lepidopteran pests. Results showed that the cotton line containing Vip3A was more efficacious against the beet Spodoptera exigua (Hübner) and fall armyworm Spodoptera frugiperda (J. E. Smith) compared to the Cry1Ab cotton line; however, the Cry1Ab cotton line was more efficacious against the tobacco budworm Heliothis virescens F. compared to the cotton line containing Vip3A. Both the Vip3A and Cry1Ab cotton lines provided similar mortality against the bollworm Helicoverpa zea (Boddie). No synergism between Vip3A and Cry1Ab was observed.

RESUMEN

Desde el año 2004 hasta el final de 2005, se evaluaron parcelas experimentales con líneas transgénicas de algodón que contienen la proteína vegetal insecticida, Vip3A; δ-endotoxin, Cry1Ab; y se evaluaron ambas Vip3A y Cry1Ab por su eficacia contra ciertas plagas lepidópteros. Los resultados mostraron que la línea de algodón que contiene Vip3A fue mas eficaz contra el gusano de ejercito de remolacha, *Spodoptera exigua* (Hübner) y el gusano cogollero, *Spodoptera frugiperda* (J. E. Smith) en comparación con la línea Cry1Ab de algodón; sin embargo, la línea Cry1Ab de algodón fue mas eficaz contra el gusano del brote de tabaco, *Heliothis virescens* F. en comparación con la línea de algodón con Vip3A. Ambas líneas de algodón (Vip3A y Cry1Ab) proveyeron una mortalidad similar contra el gusano de la belota, *Helicoverpa zea* (Boddie). Ningún sinergismo fue observado entre la Vip3A y Cry1Ab.

Advancements for insect control that utilize transgenic technology will continue to improve efficacy against many lepidopteran pests. Current and experimental cotton varieties can contain Cry1Ac alone (Bollgard®, Monsanto Ag. Co., St. Louis, MO), or they can be stacked with Cry2Ab (Bollgard® II, Monsanto Ag. Co.) or Cry1F (WideStrike®, Dow Agrosciences, Indianapolis, IN). Furthermore, a novel vegetative insecticidal protein from *B. thuringiensis* (Vip3A) stacked with Cry1Ab is currently in development (VipCot™, Syngenta Crop Protection, Greensboro, NC).

The beet armyworm, *Spodoptera exigua* (Hübner) is an occasional but serious pest of various vegetable and row crops in the mid-southern United States of America. Although larval feeding on cotton is primarily concentrated on foliage, larvae can cause devastating losses in yield (Hardee & Herzog 1997; Adamczyk et al. 1998). The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) also is a destructive migratory pest of many crops in the Western Hemisphere (Sparks 1979; Young 1979). Like the beet armyworm, this pest has the potential to damage both conventional cotton bolls and Bollgard® cotton bolls (Adamczyk et al. 1998).

Although certain lepidopteran pests of cotton are controlled by Bollgard® cotton (e.g., tobacco budworms, *Heliothis virescens* F. and pink bollworms, *Pectinophora gossypiella* (Saunders)), the Cry1Ac δ-endotoxin in Bollgard® cotton is less effective for controlling beet and fall armyworms, and bollworms, *Helicoverpa zea* (Boddie) (Adamczyk et al. 1998; Henneberry et al. 2001). Consequently, outbreaks of these pests on Bollgard® often need full application rates of foliar insecticide treatments to keep populations below economic injury levels (Hood 1997; Smith 1997). However, the addition of other Cry proteins stacked with Cry1Ac appears to have improved the efficacy (i.e., Bollgard II® and WideStrike™) against beet and fall armyworms, and bollworms (Adamczyk et al. 2001; Stewart et al. 2001; Adamczyk & Gore 2004). The purpose of the study was to examine the efficacy of Vip3A, Cry1Ab, and Cry1Ab + Vip3A traits against various Lepidoptera in laboratory bioassays and small experimental field plots.

MATERIALS AND METHODS

Field Plots

From 2004-2005, experimental transgenic cotton lines containing Vip3A (2004 and 2005), Cry1Ab (2005), or Cry1Ab stacked with Vip3A

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(2005) were planted in research plots in the Mississippi Delta (Table 1). In 2004, three different transgenic cotton lines containing the Vip3A event were planted near Stoneville, MS in early May with plots consisting of 2 rows (1.0 m centers x 10.67 m). These events (COT102, COT202 and COT203) contained different promoters driving Vip3A expression, where COT202 and COT203 contained a more robust promoter than COT102. In 2005, eight different transgenic cotton lines containing Vip3A, Cry1Ab, or Cry1Ab stacked with Vip3A events were planted in late Apr near Scott, MS with plots consisting of 4 rows (1.0 m centers × 12.20 m). In addition, 3 different Cry1Ab lines that had different insertion events (02A, 67B, and 69D) were evaluated. All plots were arranged in a randomized complete block design with each variety replicated 4 times (once in each block, except COT102 replicated twice in each block in 2004). Only insecticides not active on Lepidoptera were applied to all plots throughout the season as dictated by local management practices. All Lepidoptera (beet and fall armyworms, bollworms, and tobacco budworms) utilized in these studies were obtained from laboratory colonies maintained at the USDA, ARS, Southern Insect Management Research Unit, located in Stoneville, MS.

Efficacy of Vip3A Events (2004)

Terminal leaves or flower buds (squares, fall armyworms only) containing 3 Vip3A events were assayed for bioactivity against beet armyworms, fall armyworms, bollworms, and tobacco budworm neonates (Table 1). Squares were utilized to evaluate activity against fall armyworms because the larval stage primarily feeds on fruit (Adamczyk et al. 1998). Individual leaves or squares collected from plants that had begun to flower were placed into a Tight-Fit Lid sealing Petri dish (50 x 9 mm, BD Falcon® #351006, VWR International). A single larva was placed in a dish containing a single terminal leaf or square. Ten dishes per plot were used for bollworms, while 20 dishes per plot were used for beet armyworms and tobacco budworms. Thirty dishes per plot were used for fall armyworms. At various days after exposure to cotton tissue, larvae were prodded with a camelhair brush and considered dead if no coordinated movement was observed. Percent mortality of neonates was analyzed by REML-ANOVA, and means were separated by LSMEANS (Littell et al. 1996; PROC MIXED, SAS Institute 2001).

Because of the historic ability of the tobacco budworm to cause severe economic losses to cotton, a field experiment was conducted to further evaluate efficacy of the Vip3A events against this pest. Two 1-d-old larvae were placed on a single terminal and covered with a nylon cage to prevent interplant movement (Fig. 1). Plants were at the pre-bloom stage at the time of infestations. Ten cages were utilized per plot. After 2 d, 3 cages per plot were removed and larval mortality was recorded. The remaining cages were removed and assessed at 5 d after larval infestations. All larvae that were alive were placed in individual 1-oz soufflé cups and transported to the laboratory in a cooler containing ice. Larvae were weighed within 2 h.

TABLE 1. BIOASSAYS CONDUCTED WITH COTTON LINES IN 2004 AND 2005.

Year	Cotton Lines	Trait
2004	COT 102	Vip3A
	COT 202	Vip3A
	COT 203	Vip3A
	Coker 312	None
2005	COT 102	Vip3A
	COT 203	Vip3A
	02A	Cry1Ab
	67B	Cry1Ab
	69D	Cry1Ab
	02A x COT202	Cry1Ab + Vip3A
	67B x COT202	Cry1Ab + Vip3A
	69D x COT202	Cry1Ab + Vip3A
	Coker 312	None
	DP 393	None
	DP 491	None
	DP 493	None

Efficacy of the Vip3A, Cry1Ab, and Cry1Ab + Vip3A Traits (2005)

Terminal leaves from different cotton lines containing 2 Vip3A events, 3 Cry1Ab events, and 3 Cry1Ab stacked with Vip3A events were assayed as described above for bioactivity against



Fig. 1. Cages used to enclose tobacco budworms on cotton terminals

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beet armyworms, fall armyworms, bollworms, and tobacco budworm neonates (Table 1). Three larvae (tobacco budworms) or a single larva (all other species) were placed in a dish containing a single terminal leaf from plants that had begun to flower. Ten dishes per plot were utilized for all species, and mortality was assessed at 5 days after exposure. Percent mortality of neonates was combined by trait and analyzed by REML-ANOVA. Means were separated by LSMEANS (Littell et al. 1996; PROC MIXED, SAS Institute 2001).

Results and Discussion

Efficacy of Vip3A Events (2004)

All cotton lines containing the Vip3A event caused significantly higher mortality against all examined pests compared to conventional cotton (Figs. 2-4). COT202 and/or COT203 were significantly more efficacious than COT102 against the bollworm, tobacco budworm, and beet armyworms (Figs. 2-3), although not apparent for fall armyworms fed squares (Fig. 4). All cottons containing the Vip3A event significantly reduced the number of tobacco budworms recovered alive 5 d after infestation when caged on terminals, although no cotton caused 100% mortality (Fig. 5). In addition, the weight of larvae recovered alive at 5 d after infestation from all cotton with the Vip3A event was significantly less than those larvae recovered alive from conventional cotton. However, tobacco budworms recovered from COT202 and COT203 weighed significantly less than larvae recovered from COT102 (Fig. 6).

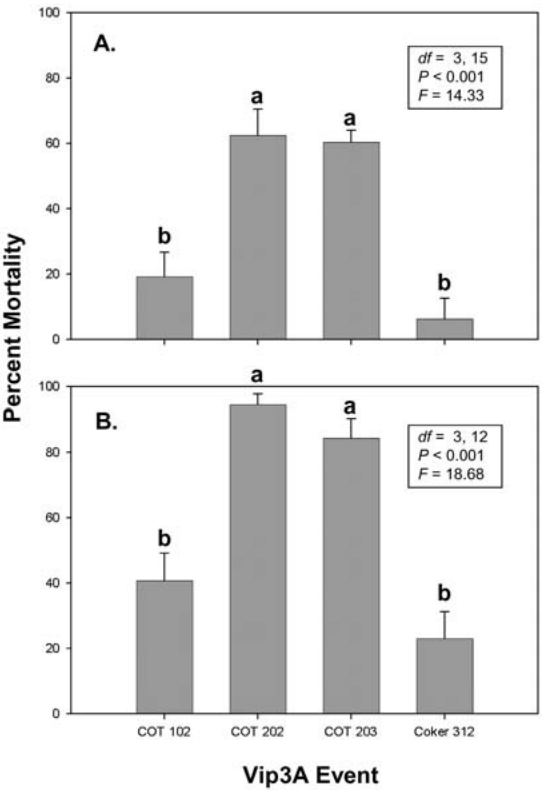


Fig. 2. Efficacy at (A) 2 d after exposure and (B) 4 d after exposure to terminal leaves containing Vip3A events against bollworm larvae. Coker 312 is a conventional cotton variety. Bars (Mean ± SE) with a common letter are not significantly different ($\alpha = 0.05$) according to LSMEANS.

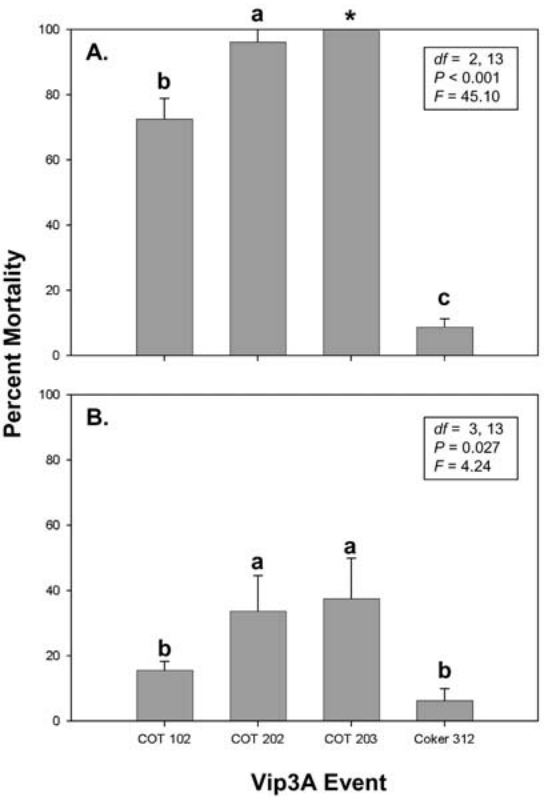


Fig. 3. Efficacy at 7 d after exposure to terminal leaves containing Vip3A events against beet armyworm (A) and tobacco budworm (B) larvae. Coker 312 is a conventional cotton variety. 100% mortality was observed for beet armyworms fed cotton containing COT203; therefore, it was excluded in the ANOVA. Bars (Mean ± SE) with a common letter are not significantly different ($\alpha = 0.05$) according to LSMEANS.

Efficacy of the Vip3A, Cry1Ab, and Cry1Ab + Vip3A Traits (2005)

All cotton lines containing the Vip3A or Cry1Ab trait caused significantly higher mortality against tobacco budworm larvae than conventional cotton (Fig. 7A). In addition, cotton lines

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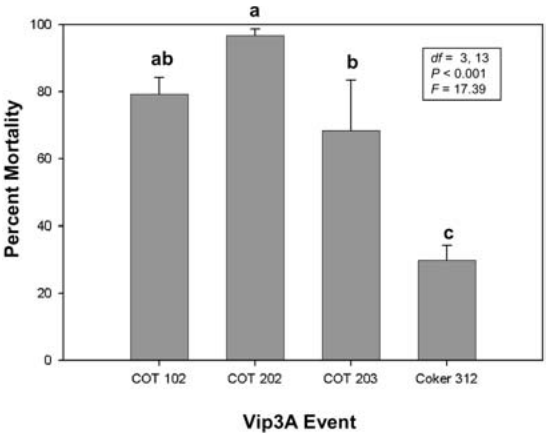


Fig. 4. Efficacy at 3 d after exposure to squares containing Vip3A events against fall armyworm larvae. Coker 312 is a conventional cotton variety. Bars (Mean ± SE) with a common letter are not significantly different ($\alpha = 0.05$) according to LSMEANS.

containing the stacked Cry1Ab + Vip3A trait caused significantly higher mortality than cotton containing just the Vip3A trait. There were no significant differences in larval mortality when tobacco budworm larvae were fed with cotton containing Cry1Ab alone or stacked with Vip3A.

In contrast, lines that contained just the Vip3A trait were equally efficacious against bollworm larvae compared to lines containing just the Cry1Ab trait or lines containing the stacked Cry1Ab + Vip3A trait (Fig. 7B). Similar to the data for tobacco budworms, all cotton lines containing Vip3A or Cry1Ab traits caused significantly higher mortality against bollworm larvae compared to conventional cotton.

For both beet and fall armyworms, cotton lines containing the Vip3A trait caused significantly higher morality compared to cotton lines containing just the Cry1Ab trait (Fig. 8). All cotton lines containing Vip3A or Cry1Ab traits caused significantly higher mortality against beet and fall armyworm larvae compared to conventional cotton. There were no significant differences in larval mortality when beet and fall armyworm larvae were fed cotton that contained Vip3A alone or stacked with Cry1Ab.

The efficacy of Vip3A and Cry1Ab against Lepidoptera is species-specific. Our study shows that the cotton lines containing Vip3A are more efficacious against beet and fall armyworms compared to the Cry1Ab cotton lines, while the opposite relationship is true for tobacco budworms. Previous studies have shown that certain Lepidoptera, such as tobacco budworms, are more susceptible to Cry1Ac compared to bollworms and fall armyworms, while Cry1F provides greater efficacy against fall armyworms compared to Cry1Ac (MacIntosh et al. 1990; Adamczyk et al. 1998; Adamczyk & Gore 2004). In addition, no additive or synergistic relationship between the Vip3A and Cry1Ab traits in these particular cotton lines was observed. This is similar to what was reported for the 2 Cry proteins in WideStrike® cotton (Adamczyk & Gore 2004). Parker & Livingston (2005) reported that private industry is developing a transgenic cotton trait containing a Vip3A trait stacked with Cry1Ab trait (VipCot™). This would provide a much better spectrum of Lepidopteran control than one with the traits expressed individually.

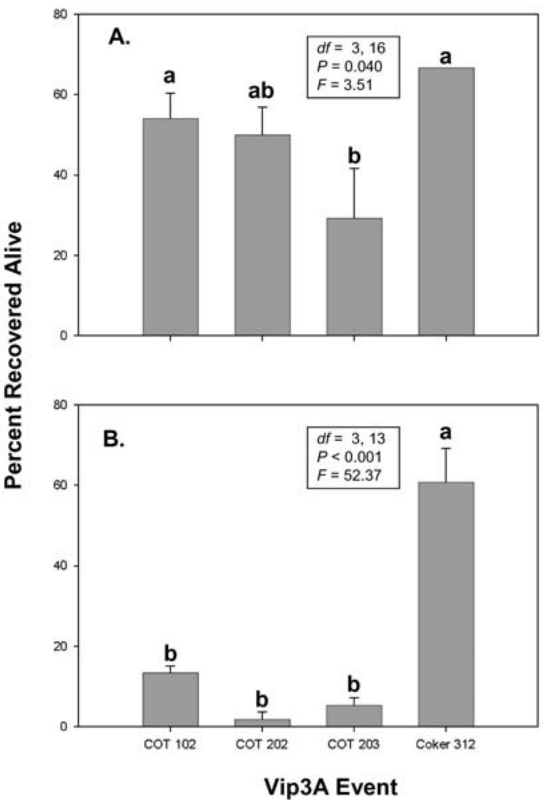


Fig. 5. Tobacco budworms recovered alive at (A) 2 d and (B) 5 d after being caged on terminal portions of cotton plants containing Vip3A events. Coker 312 is a conventional cotton variety. Bars (Mean ± SE) with a common letter are not significantly different ($\alpha = 0.05$) according to LSMEANS.

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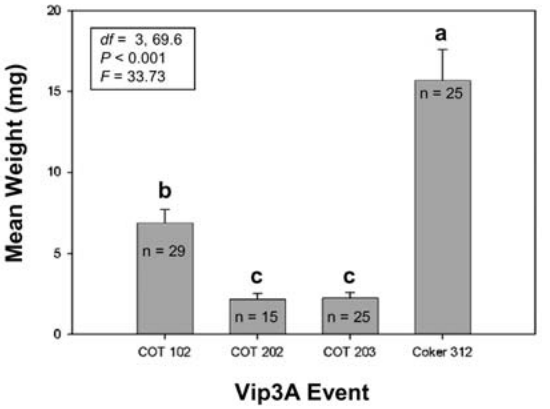


Fig. 6. Weight of tobacco budworms recovered alive 5 d after being caged on terminal portions of cotton plants containing Vip3A events. Number (n) of larvae weighed per Vip3A event. Coker 312 is a conventional cotton variety. Bars (Mean ± SE) with a common letter are not significantly different ($\alpha = 0.05$) according to LSMEANS.

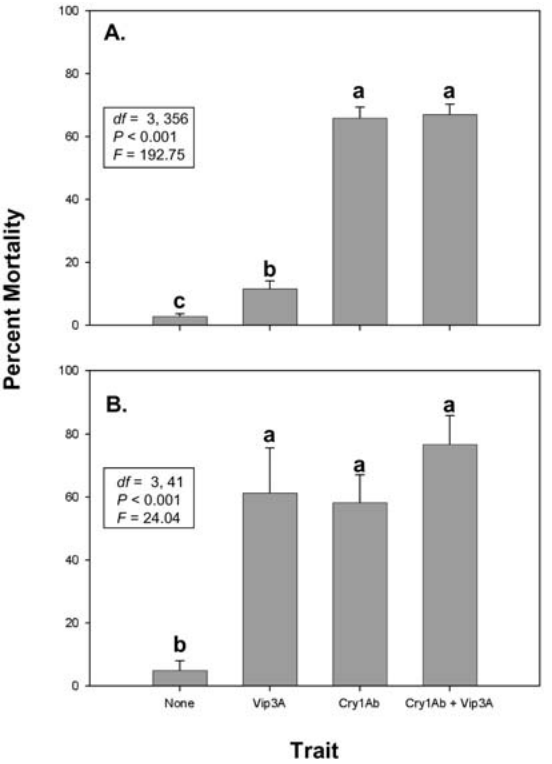


Fig. 7. Efficacy at 5 d after exposure to terminal leaves containing various transgenic traits (see Table 1, 2005) against tobacco budworm (A) and bollworm (B) larvae. Bars (Mean ± SE) with a common letter are not significantly different ($\alpha = 0.05$) according to LSMEANS.

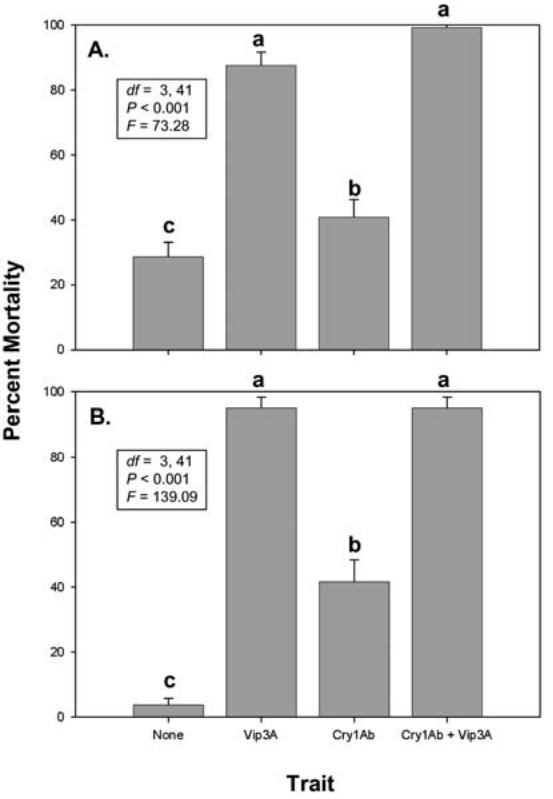


Fig. 8. Efficacy at 5 d after exposure to terminal leaves containing various transgenic traits (see Table 1, 2005) against beet (A) and fall armyworm (B) larvae. Bars (Mean ± SE) with a common letter are not significantly different ($\alpha = 0.05$) according to LSMEANS.

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