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FIELD STUDIES OF VIPCOTTM SUPPORT HIGH DOSE EFFICACY TOWARDS TBW, HELIOTHIS VIRESCENS

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Abstract

Artificial infestations of very high numbers of tobacco budworm, *Heliothis virescens*, larvae demonstrated that the Vip3A protein expressed in VipCot™ cotton meets the criteria for high-dose as defined by the EPA Scientific Advisory Panel. The high dose expression of Vip 3A will be an integral part of the proposed Insect Resistance Management strategy for VipCot™.

Introduction

The commercialization of transgenic cottons containing insecticidal Cry proteins derived from *Bacillus thuringiensis* (Berliner) have significantly improved growers ability to control certain insect pests in cotton. Among the most economically important of these pests are the tobacco budworm, *Heliothis virescens* (Fabricius), and the bollworm, *Helicoverpa zea* (Boddie). Transgenic Bt cottons that are currently commercially available include Bollgard ® expressing Cry1Ac, Bollgard II® expressing Cry 1Ac plus Cry 2Ab and Wide Strike® expressing Cry1Ac plus Cry 1F. Although Bollgard® cotton is extremely effective in controlling tobacco budworm, its limited spectrum and reduced activity towards bollworm mandates that growers add insecticide to their overall lepidopteran insect control program to avoid yield losses (Bacheler and Mott 1997; Burd *et al.* 1999; Layton *et al.* 1997, 1998, 2000; Leonard *et al*. 1997, 1998). The introduction of two-gene transgenic cottons, BollgardII® and WideStrike®, has significantly broadened the spectrum of activity of Bt cottons to include armyworm and looper species. Although BollgardII® cottons tend to exhibit higher bollworm activity compared to the single gene Bollgard®, they are not immune to damage under high bollworm populations (Jackson *et al.* 2004).

VipCot™, Syngenta's first transgenic offering in the cotton market, is expected to be commercially release in the near future. Vip3A, the insecticidal protein expressed in VipCot™, not only represents a novel mode of action (Yu *et al.* 1997, Lee *et al.* 2003) in transgenic cottons, but it also exhibits a spectrum of activity (Estruch *et al.* 1996, Cook *et al.* 2003, Cloud *et al.* 2003, Mascarenhas *et al.* 2003, Mascarenhas 2004) comparable to that of the two-gene Bt cottons. Investigations underway suggest that cross-resistance of Vip3A to Cry proteins is unlikely (Chen and Lee, 2005), which may provide a significant benefit to an overall IRM strategy involving a landscape environment with multiple traits. Upon commercialization of VipCot™, an IRM strategy based on high dose insecticidal protein expression and structured refuge will be implemented to ensure good stewardship of this technology, as well as other technologies in the market place.

Reported here are results of several field studies designed to assess if Vip3A expressed in VipCot™ events meets one of the high dose criteria established by the EPA.

Methods and Materials

Two VipCot™ events, Cot 202 and Cot 203, were evaluated for their ability to meet the high dose criteria number four set by the EPA Scientific Advisory Panel (SAP). Locations in which studies were conducted and the particular

events evaluated are listed in Table 1. VipCot™ cotton was artificially infested with tobacco budworm eggs, which were obtained form the Southern Insect Management Laboratory in Stoneville, MS 24 to 36 hours prior to artificial infestation. Eggs were mixed into a xathan gum solution and sprayed to the terminal area of cotton plants utilizing a conventional CO_2 backpack sprayer. Eggs were sprayed through a flat fan 8006 nozzle at approximately 10 psi. Unreplicated, solid blocks of approximately 3,500 to 5,000 plants of VipCot™ (Cot 202 and Cot 203), as well as a smaller block (350 to 500 plants) of non-transgenic Coker 312 were utilized for the infestation. If populations of natural enemies were deemed to be sufficiently high to interfere with infestation, the study area was over sprayed with acephate (Orthene®) at 0.5 lb ai/A 24 to 48 hours before scheduled infestation. The non-transgenic Coker 312 cotton block was used to estimate the infestation technique effectiveness and to determine field fitness of the tobacco budworm strain utilized in these studies. At each location, two to three artificial infestations were made to both VipCot™ and Coker cotton at different crop developmental stages. The first of these infestations was timed at around 2 to 3 weeks after pinhead square and the last was at early boll maturation. The method by which eggs hatch was estimated varied by location, but in general hatch was estimated by collecting several leaves containing eggs from Coker 312 plants and placing them into petri dishes. Eggs on the collected leaves were counted and two to three days later, successful larval eclosion was assessed. One half of all the artificially infested plants were thoroughly sampled 10 days after infestation. Although sampling every single fruiting structure was prohibitive given the sampling regime magnitude (approximately 1,250 plants per location, event and sampling date), a general overview of each plant was made in an attempt to locate sites within the canopy where larval feeding may have occurred. Once these possible "feeding sites" were identified, approximately two squares per plant were evaluated for signs of larval damage and presence of live larvae. This sampling regime generally resulted in squares being sampled from the top, middle and lower 1/3 of the plant canopy. In total, approximately 2,500 VipCot™ squares were sampled at each location, event and sampling date. In the smaller Coker 312 block, approximately 250 squares were sampled at each location, event and sampling date. VipCot™ squares containing live larvae were tagged for further observation four days later.

Results

Across all locations and infestation cycles, a total of 291,200 and 738,000 eggs were sprayed onto Cot 202 and Cot 203, respectively. At the one location in which Cot202 was evaluated, a conservative hatch rate of 36% resulted in approximately 105,069 larvae infested onto Cot202 plants. With a conservative average hatch rate of 48.6% across the three locations in which Cot 203 was evaluated, approximately 358,668 larvae were infested onto Cot 203 plants. Due to severe inclement weather at the Vero Beach site, the last infestation cycle of 34,395 larvae was not assessed. Therefore, the final number of established larvae for which recovery assessments were made was 320,249 for Cot 203, respectively.

Larval Mortality and Square Damage to Cot 202

VipCot™ event Cot 202 was evaluated in a study near Winnsboro, Louisiana. A total of 105,069 neonate larvae successfully hatched onto VipCot™ plants (Table 2). A smaller cohort of 10,393 larvae was also established onto non-transgenic Coker 312 plants. A total of 48 larvae, ranging from fourth to fifth instar, were recovered from Coker 312 cotton 10 days after infestation. The fitness of the strain utilized was determined by the ability of the larvae to cause damage to cotton squares under field conditions. The average percent damaged square observed in Coker plants was 24.4%. Of the 105,069 larvae established onto Cot 202, only 22 were recovered 10 days after infestation. Because most of the observed larvae on Cot 202 ranged from a first to a early third instar, the structures where they were found were tagged to investigate if these larvae would continue to develop. At 14 days after infestation, the tagged square and adjacent fruiting structures were sampled and only 2 larvae remained on Cot 202 plants. Larval recovery at 14 days after infestation was not assessed in the Coker plots because many of the infested larvae had worked their way to the bottom of the canopy and begun to search for a pupation site in the soil, which would have impacted the accuracy of the assessment. Percent damage square observed in Cot 202 averaged 0.5% across the various infestation cycles and in many cases, only superficial damage to the bract or calyx of squares was noted.

Larval Mortality and Square Damage to Cot 203

VipCot™ event Cot 203 was evaluated in Texas, Mississippi and Florida. At each of these respective sites, a total of 119,860, 117,000 and 83,389 neonate larvae successfully hatched onto VipCot™ plants (Table 3). A smaller cohort of larvae (9,740 in Texas, 11,000 in Mississippi and 15,709 in Florida) was also established onto non-transgenic

Coker 312 plants. Across all three locations, a total of 424 larvae, ranging from fourth to fifth instar, were recovered from Coker 312 cotton 10 days after infestation. Percent damage squares observed in Coker plants ranged from 23 to 69% across the three locations. Of the 320,249 larvae established onto Cot 203 across the three locations, only 76 and 6 larvae were recovered at 10 and 14 days after infestation, respectively. As with Cot 202 studies, larval recovery at 14 days after infestation was not assessed in the Coker plots. Percent damage square observed in Cot 203 ranged from 0.5 to 2.8% across the three locations.

Discussion

The level of insecticidal protein (Vip3A) expression in Cot 202 and Cot 203 was highly efficacious in controlling very high populations of tobacco budworm. As a result of its high insecticidal protein expression, VipCot™ provides excellent protection against larval feeding. Averaged across the three locations, Cot 203 exhibited 2% damage squares, while damage to Coker squares averaged 36.7%. Similar results were observed with Cot 202. Because a smaller block of Coker 312 was utilized in these studies to logistically be able to handle the intensive sampling regime of VipCot™ squares (2,500 per location, event and sampling date), the total number of larvae infested onto Coker plants was 10.1 and 8.9 times less than in Cot 202 and Cot 203, respectively. Had the Coker blocks been sufficiently large to accommodate the same infestation regime as the VipCot™ blocks, the expected number of larvae recovered from Coker plants at 10 days after infestation would have ranged from 485 and 3,752. By contrast, the total numbers of larvae recovered from Cot 202 and Cot 203 after the same length of exposure were 22 and 76 larvae, respectively. The subsequent sampling of the tagged squares and adjacent fruiting structures revealed substantial additional larval mortality in both VipCot™ events at 14 days after infestation. The fact that the few larvae remaining on VipCot™ 14 days after infestation were substantially smaller (½ inch in length or less) compared to those observed earlier on Coker plants (greater than 1-1/8 inch fifth instar, entering pupation), suggest that generational survival in VipCot™ plants is unlikely, given the fitness penalties observed at the larval stage. Results from these studies demonstrates that both VipCot™ events not only provide excellent plant protection, but they express the Vip3A protein at sufficiently high levels to be considered high dose towards tobacco budworm, thus satisfying the EPA criteria number four. The demonstrated high dose of VipCot™ coupled with required refuge should allow VipCot™ to be commercialized under the already established IRM guidelines, which will avoid complexity in the marketplace and promote grower acceptance of the technology, as well as compliance with guidelines.

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Table 1. Field sites and specific event (s) evaluated in 2004.

	Cot 202	Cot 203
Trial Locations		
Beasley, Texas		√
Winnsboro, Louisiana	√	
Leland, Mississippi		√
Vero Beach, Florida		√

Table 2. Demonstration of VipCot™ event Cot 202 as high dose towards tobacco budworm in 2004.

Location:	Winnsboro, LA	
Genotype:	Coker 312	Cot 202
No. Infested Larvae	10,393	105,069
No. Squares Sampled	750	7,519
No. Recovered Larvae 10 Days	48	22
No. Recovered Larvae 14 Days	--	2
No. Damaged Square	183	38
Percent Damaged Squares	24.4	0.51

Table 3. Demonstration of VipCot™ event Cot 203 as high dose towards tobacco budworm in 2004.

Location:	Beasley, TX		Leland, MS		Vero Beach, FL	
Genotype:	Coker 312	Cot 203	Coker 312	Cot 203	Coker 312	Cot 203
No. Infested Larvae	9,740	119,860	11,000	117,000	15,709	83,389
No. Squares Sampled	1,000	10,000	750	7,500	2,500	5,000
No. Recovered Larvae 10 Days	70	10	268	54	86	12
No. Recovered Larvae 14 Days	--	0	--	6	--	0 [a]
No. Damaged Square	233	49	518	213	750	134
Percent Damaged Squares	23.3	0.49	69.1	2.84	30.0	2.68

[a] Assessment of larval mortality 14 days after infestation is based on a single infestation cycle of 61,020 larvae.