# BOLLGARD III IMPACT ON DAMAGE AND SURVIVORSHIP OF FALL ARMYWORM ON COTTON FRUITING FORMS

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## Abstract

Laboratory studies evaluated fall armyworm, *Spodoptera frugiperda* (J. E. Smith), damage and survivorship on fruiting forms of transgenic *Bacillus thuringiensis* (Bt) cotton plants. Third instars from a laboratory colony were offered freshly harvested flower buds (squares) or bolls of conventional non-Bt, Bollgard II® (Cry1Ac + Cry2Ab), and Bollgard III (Cry1Ac + Cry2Ab + Vip3A) cotton lines in no-choice tests. Plant tissue was replaced every two-three days and a record of survivorship was recorded at the same intervals. Damage to fruiting forms and fall armyworm larval survivorship were significantly affected by Bt lines compared to that observed on conventional non-Bt cotton lines. Bollgard III tissue had significantly fewer damaged fruiting forms and lower larval survivorship compared to that on Bollgard II® tissue.

## Introduction

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is an occasional pest of cotton, occurring sporadically throughout the growing season and across the cotton-producing regions. This insect pest is unable to enter diapause, forcing it to migrate into U.S. cotton-growing regions each year from warmer environments such as South Florida, South Texas, Mexico, Caribbean islands, or Central America (Sparks 1979, Knipling 1980, Ashley 1979, Adamczyk et al. 1997, Adamczyk 1998). Insecticide treatments commonly effective against primary caterpillar pests of cotton such as the bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), generally provide inconsistent control of fall armyworm (Adamczyk et al. 1997). In addition, the dispersal of fall armyworm larvae in the lower strata of the plant canopy and poor deposition of insecticides in this plant region are responsible for lower insecticide efficacy against this pest.

The first transgenic *Bacillus thuringiensis* var. *kurstaki* (Berliner) (Bt) cotton cultivars containing insecticidal crystal (Cry) proteins, known as Bollgard®, became available in the U.S. during 1996 (Jackson et al. 2005). The protein in Bollgard®, Cry1Ac, is highly toxic to tobacco budworm and pink bollworm, *Pectinophora gossypiella* (Saunders) (Jackson et al. 2005). Cry1Ac, however, is not as effective against the bollworm and other cotton Lepidopteran pests (Stewart et al. 2000). Bollgard II® (Cry1Ac + Cry2Ab) and WideStrike™ (Cry1Ac + Cry1F) were commercially released in 2003 (Monsanto) and 2005 (Dow AgroSciences), respectively (Siebert et al. 2008). Bollgard II® combines tobacco budworm control with significantly improved bollworm control. WideStrike™ provides control of many of the same target pests as Bollgard II®, but the addition of the Cry1F protein improves efficacy against secondary Lepidopteran pests (Willrich et al. 2005, Tindall et al. 2006).

Monsanto is developing the next generation of Bt technology in cotton, which utilizes a novel combination of Bt proteins. Bollgard III technology incorporates the Cry1Ac and Cry2Ab endotoxins found in Bollgard II® cotton varieties with the addition of a Bt exotoxin (Vip3A). The Vip3A protein is unique in both structure and mode of action compared to the Cry proteins currently used in other commercial Bt cotton varieties (Leonard et al. 2005).

The efficacies of Bollgard®, Bollgard II®, and WideStrike™ cotton lines have been evaluated in numerous studies against primary cotton pests such as heliothines and pink bollworm (Henneberry et al. 2001, Sims et al. 2002, Haile et al. 2004, Adamczyk and Mahaffey 2007, Bommireddy and Leonard 2008) . Considerable information has recently become available on the performance of these Bt technologies against occasional insect pests such as fall armyworm and other *Spodoptera spp*. However, for the Bollgard III traits, no results have been reported against these pests. Therefore, the objective of this report is to summarize the results of preliminary laboratory trials on damage and survivorship of fall armyworm on cotton fruiting forms expressing multiple Bt proteins.

## Materials and Methods

No-choice laboratory trials were performed at the LSU AgCenter's Macon Ridge Research Station (MRRS) near Winnsboro, LA, during 2010. The transgenic traits (cotton lines) in these tests were a Bollgard III® advanced breeding line and a Bollgard II® background line. A conventional non-Bt line was tested as a negative control to standardize larval survival for a non-Bt cotton genotype and for comparison to each Bt line. Field plots were planted on 4 June 2010 and managed with recommended agronomic and IPM strategies to optimize plant development and production of fruiting forms.

The fall armyworm colony originated from field collections in cotton (fall, 2005) and field corn (summer, 2006 & 2008). It has been maintained as a laboratory colony on meridic diet according to previously described methods (Adamczyk et al. 1998). This fall armyworm colony has been validated according to analysis of the mitochondrial *Cytochrome oxidase I* (COI) gene as the corn-cotton strain by (Rod Nagoshi, USDA-ARS, Gainesville, FL; personal communication).

The procedures for infesting fall armyworms on cotton tissues were adapted from those used in similar studies by Adamczyk et al. (1998) and Bommireddy and Leonard (2008). For each date of infestation and subsequent observations, all floral buds (squares) and bolls were immediately harvested from field plots, debracted, gently washed, and placed into cups. Every attempt was made to collect these structures from first position sites on sympodial branches. One larva (third-instar, 30-45 mg) was removed from the laboratory colony and placed in a 29.6 ml plastic cup containing three squares. In a similar manner, a third instar was placed in a 104 ml plastic cup containing one boll (7-10 d after anthesis). Cups for square and boll infestations were sealed with a plastic lid to prevent larval escape and to reduce desiccation of plant tissue. Squares and bolls were exchanged on a schedule of one to three days and five to seven days, respectively, after infestation or more often when daily examination of the cups indicated the plant tissue was deteriorating or had been fully consumed by the larva. Three replicates, each with 30 larvae (total n = 90 larvae per cotton line) were evaluated on squares. Four replicates, each with 20 larvae (total n = 80 larvae per cotton line) were evaluated on bolls. Cumulative larval survivorship was recorded daily. Data on penetrated fruiting forms was recorded only for the initial offering of fresh tissue. The endpoint of experiments was defined by 100% mortality on the Bollgard III line and occurred at 8 d after infestation (DAI) on both squares and bolls. Fruiting form penetration and survivorship data were analyzed using a randomized complete block design (replicates = infestation events) using PROC GLM. Cotton trait means were separated according to LSD (SAS Institute 2004).

## Results and Discussion

Fall armyworm larvae readily consumed squares of the non-Bt control cotton line. Fall armyworm larvae were capable of penetrating 76%, 60%, and 45% of squares on non-Bt, Bollgard II®, and Bollgard III, respectively (Table 1). Larval survivorship on non-Bt cotton squares was 78% at 8 DAI. On Bollgard II® squares, survivorship was 49% at 8 DAI. On Bollgard III squares, complete (100%) mortality of fall armyworm occurred at 8 DAI.

Third instars also actively fed on bolls of the non-Bt line. Fall armyworm larvae were capable of penetrating 47%, 18%, and 3% of bolls on non-Bt, Bollgard II®, and Bollgard III, respectively (Table 1). Larval survivorship on non-Bt cotton bolls was 49% at 8 DAI. On Bollgard II® bolls, larval survivorship was 23% at 8 DAI. On Bollgard III bolls, complete (100%) mortality was observed at 8 DAI.

**Table 1. Fruiting form damage and survivorship of fall armyworm infested on selected cotton lines 8 DAI in no-choice tests.**

| | Squares | | Bolls | |
|---|---|---|---|---|
| Cotton Line | Square Penetration | Larval Survivorship | Boll Penetration | Larval Survivorship |
| Conventional non-Bt | 76% a | 78% a | 47% a | 49% a |
| Bollgard II® | 60% b | 49% a | 18% b | 23% b |
| Bollgard III | 45% c | 0% b | 3% c | 0% c |

Means in the same column followed by different letters are significantly different according to LSD (*P=0.05*).

Fall armyworm damage and survivorship on Bollgard III squares and bolls was significantly lower compared to that on the conventional non-Bt line. Bollgard II® significantly reduced fall armyworm damage to squares and bolls as well as larval survivorship on bolls compared to the non-Bt. In addition, Bollgard III significantly differed from Bollgard II® for fruiting form injury and larval survivorship.

The results of the present study should be considered preliminary and no definitive conclusions are proposed by the authors. These observations represent the first attempt to characterize Bollgard III activity against fall armyworm in laboratory or field trials. Further studies will be attempted in both laboratory and field trials during 2011. The final results generated at the conclusion of these experiments should better describe the activity of Bollgard III cotton traits against fall armyworm. Cotton producers will then have additional information to use in the selection of the most appropriate cultivar and Bt trait combination.

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