

INFLUENCE OF DUAL-GENE BT CORN ON BOLLWORM, *HELICOVERPA ZEA*, SURVIVORSHIP ON BOLLGARD II COTTON

Ben Von Kanel

Angus Catchot

Jeff Gore

Fred Musser

Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology

Mississippi State University

Starkville, MS

Ryan Jackson

USDA-ARS, Stoneville, MS

Abstract

A landscape survey was conducted to evaluate the number of bollworms developing on Genuity VT3 PRO corn that contribute the overall populations infesting cotton in late summer. The overall bollworm population was very low in the majority of the corn acreage throughout the summer which lessened the number of bollworms infesting cotton. Larvae were collected from both VT3 PRO and non-Bt corn and allowed to pupate in order to arrange reciprocal and back crosses. Progeny from each cross were subjected to tissue-overlay bioassays using lyophilized Bollgard II cotton tissue. Progeny resulting from bollworm moths reared on VT3 PRO corn had a higher LC50 compared to moths whose parents were reared on non-Bt corn.

Introduction

Bollworm, *Helicoverpa zea* (Boddie), has been collected from a total of 238 plant species within 36 plant families (Kogan et al. 1989). Of these, field corn is the most preferred and the most suitable host (Isley 1942, Gore et al. 2003). Larvae complete development more rapidly and adults are more fecund when reared on field corn compared to other hosts or artificial diet. The most damaging outbreaks in cotton occur where corn and cotton are in comparable acreage. However, damage to cotton is the most common complaint because it has never been economically advantageous to treat field corn in order to control bollworm (Isley 1926). Silking corn is largely favored during early summer but as corn begins to mature, bollworm populations begin to transition into cotton (Lincoln 1972). The first transgenic corn and cotton varieties utilizing *Bacillus thuringiensis* (Bt) were implemented for control of tobacco budworm, *Heliothis virescens* (Fabricius), in cotton and several corn borer species attacking field corn. Bollworm susceptibility has been highly variable (Greenplate et al. 1998, Adamczyk et al. 2001) and researchers have begun to notice an increase in the frequency of bollworm outbreaks in Bt cotton. Trait packages in both corn and cotton containing two or more Bt genes utilize the same or similar proteins in both corn and cotton (Table 1).

Table 1. Bt corn and cotton trait packages in commercial production for use against bollworm.

Trait Packages (abbreviations)*	Lepidoptera Active Traits
Cotton	
Bollgard II	Cry1Ac + Cry2Ab
Widestrike	Cry1Ac + Cry1F
Corn	
VT Double PRO (VT2P*), VT Triple PRO (VT3P*)	Cry1A.105 + Cry2Ab
Smartstax (GENSS* or SSX*)	Cry1A.105 + Cry2Ab + Cry1F
Agrisure Viptera 3110, Agrisure Viptera 3111	Cry1Ab + VIP3A

The majority of corn acreage in Mississippi is planted to varieties containing at least one Bt protein. Furthermore, a large percentage of the bollworms infesting Bt cotton completed at least one generation in field corn (Jackson et al. 2008, Head et al. 2010). These factors are cause for concern about the potential selection pressure placed on bollworm populations completing development in Bt field corn and then transitioning into Bt cotton. To address these concerns, the objective of this research was to evaluate the contribution of bollworms in Genuity VT3 PRO corn to the overall landscape and to determine the susceptibility of bollworms collected from Non-Bt and Genuity VT3 PRO field corn, and their reciprocal crosses on Bollgard II cotton.

Materials and Methods

A minimum of 20 Genuity® VT3 PRO™ corn fields (expressing Cry1A.105 and Cry2Ab2) were surveyed for bollworm density and subsequent kernel damage in the Delta and Hills region of Mississippi. At least 10 non-Bt fields within 0.5mi proximity of the VT3 Pro surveyed fields were also evaluated in order to attain a comparison of larval and egg densities. The number of eggs and larvae was recorded along with the size of each larvae by categorizing larvae as small (<0.25in) or large (>0.25in). The survey began at the R1 growth stage (silking) and terminated approximately at the R4, or dough stage. Fields were scouted weekly by examining a minimum of 100 corn ears. Corn silks were examined for the presence of eggs and/or larvae. If no eggs or larvae are observed on exterior silks, ear leaves were folded back in order to examine the ear for larvae. Any larvae observed that were 0.25in or greater were collected and placed on artificial diet.

Collected Bollworm larvae from Non-Bt and Genuity VT3 PRO field corn were placed on artificial diet, and stored in a rearing facility at Mississippi State University. Pupae were sexed by determining the presence of a ventral v-shaped suture on the females and two circular pads near the tip of the abdomen in the males. After segregating pupae by sex, backcrosses were done with each colony. Additionally, reciprocal crosses were done to determine if bollworm ability to survive on a Bt host is sex-linked. The following crosses were done:

Non-Bt (F*) x Non-Bt (M)

Non-Bt (F) x VT3P (M)

VT3P (F) x Non-Bt (M)

VT3P (F) x VT3P (M)

*Denotes the sex of pupa

Non-Bt and Bollgard II leaf tissue was collected in 1 gal zip-lock bags and placed in a -80°F freezer for approximately 48 hr. Tissue was then lyophilized and ground until dry powder would pass through a 40-mesh sieve. For bioassay, 100 mg of powder was diluted with 5.0 ml of 0.2% agar solution to make a 20 mg/ml stock solution. Further dilutions were performed from the stock solution to develop eight treatment concentrations to apply to artificial diet. Bioassay arenas were arranged by adding 0.5 ml of warm artificial diet to each well in a 128-well bioassay tray and allowed to cool at room temperature. Each well then had 50 µl of one of the treatment concentrations pipetted onto the diet surface for a total of 16 wells per concentration for each tray. Trays were again allowed to dry at room temperature. Once samples were dry, each well received one neonate larvae (16 larvae per concentration) with one tray of larvae per cross for each tissue type. Wells were then covered with perforated, adhesive tray covers and placed in a rearing room at 84°F. Larval mortality was rated seven days after initiation. Mortality was assessed by determining larvae that had not molted into the second instar (larvae weighing <10 mg). Bioassay data were analyzed using probit analysis (Proc Probit SAS version 9.2). Fiducial limits that did not overlap were considered significantly different.

Results

Larval densities in both non-Bt and VT3 PRO corn indicate bollworm pressure for much of the state was relatively low in ear-stage field corn (Fig. 1). Bollworm assay data from 2011 illustrate progeny resulting from females reared on VT3 PRO corn had a higher LC50 on Bollgard II cotton with the paternal constituent contributing nothing with respect to survivorship (Fig. 2). In 2012, assay data was similar, however, the only cross that had a significantly higher LC50 on Bollgard II cotton was the backcross from which both parents were reared on VT3 PRO corn (Fig 3).

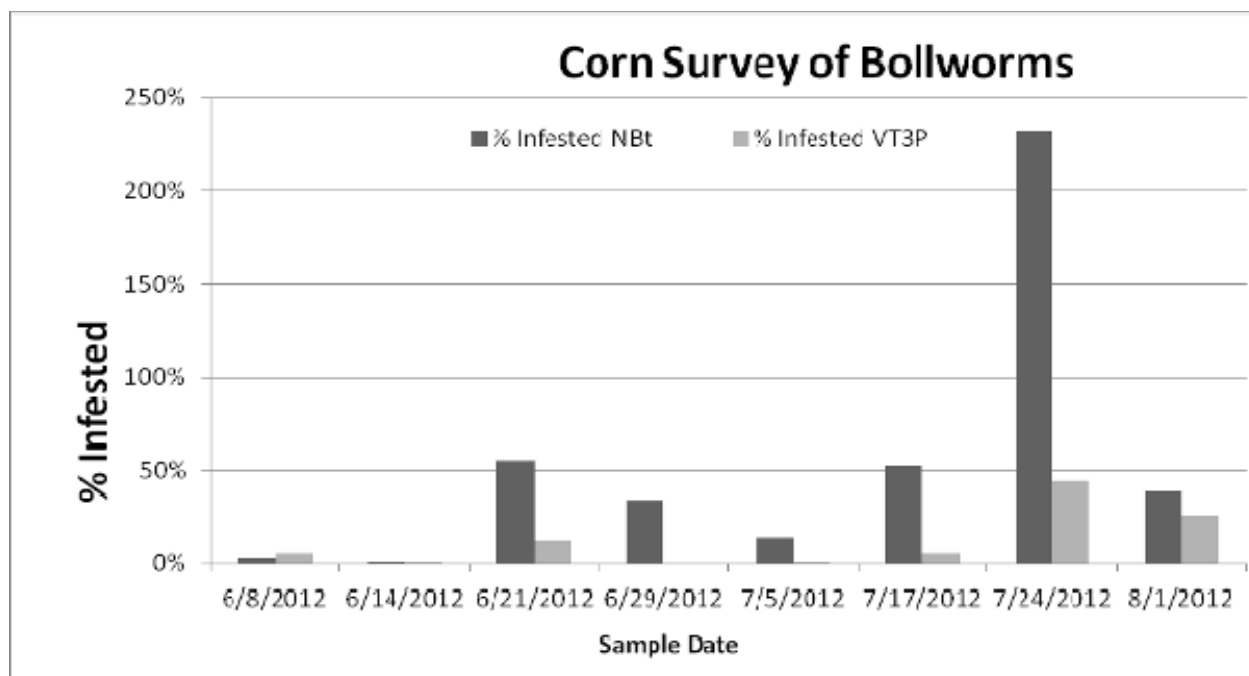


Figure 1. Landscape Survey of Bollworm Density in VT3 PRO and Non-Bt Field Corn.

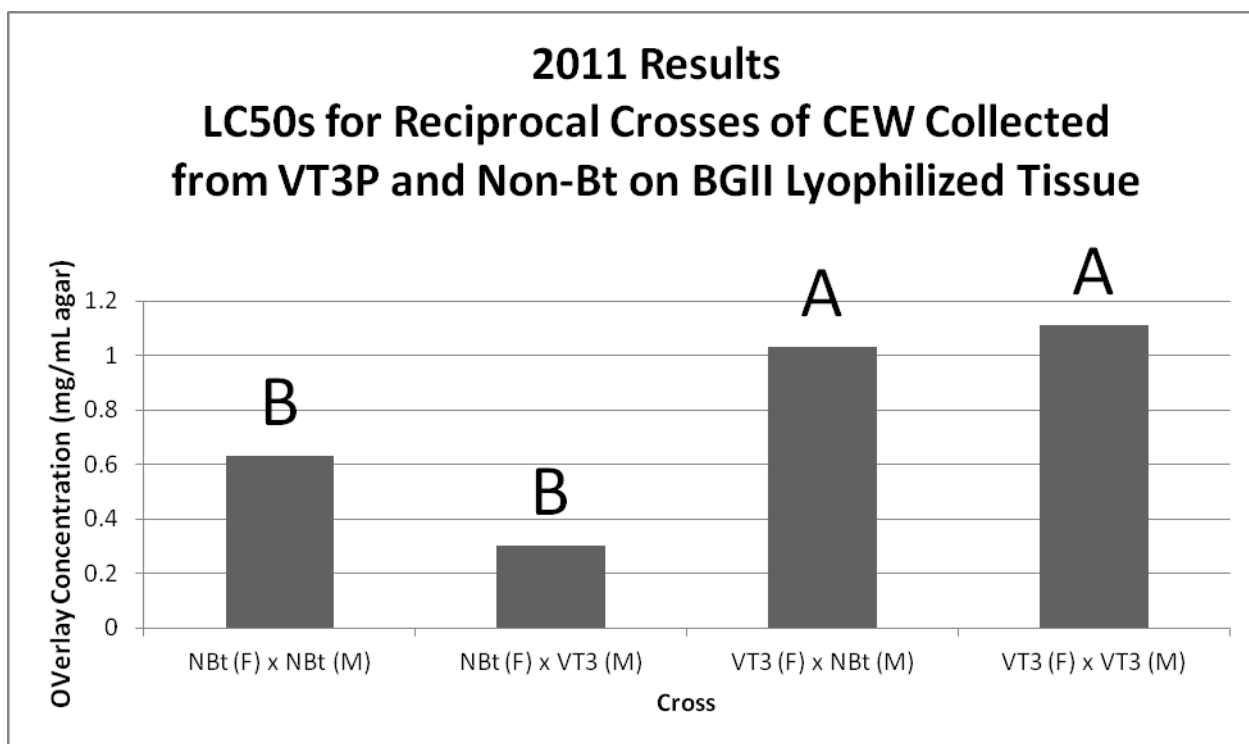


Figure 2. LC50s for Progeny Resulting from VT3 PRO and Non-Bt Field Corn on Bollgard II cotton in 2011.

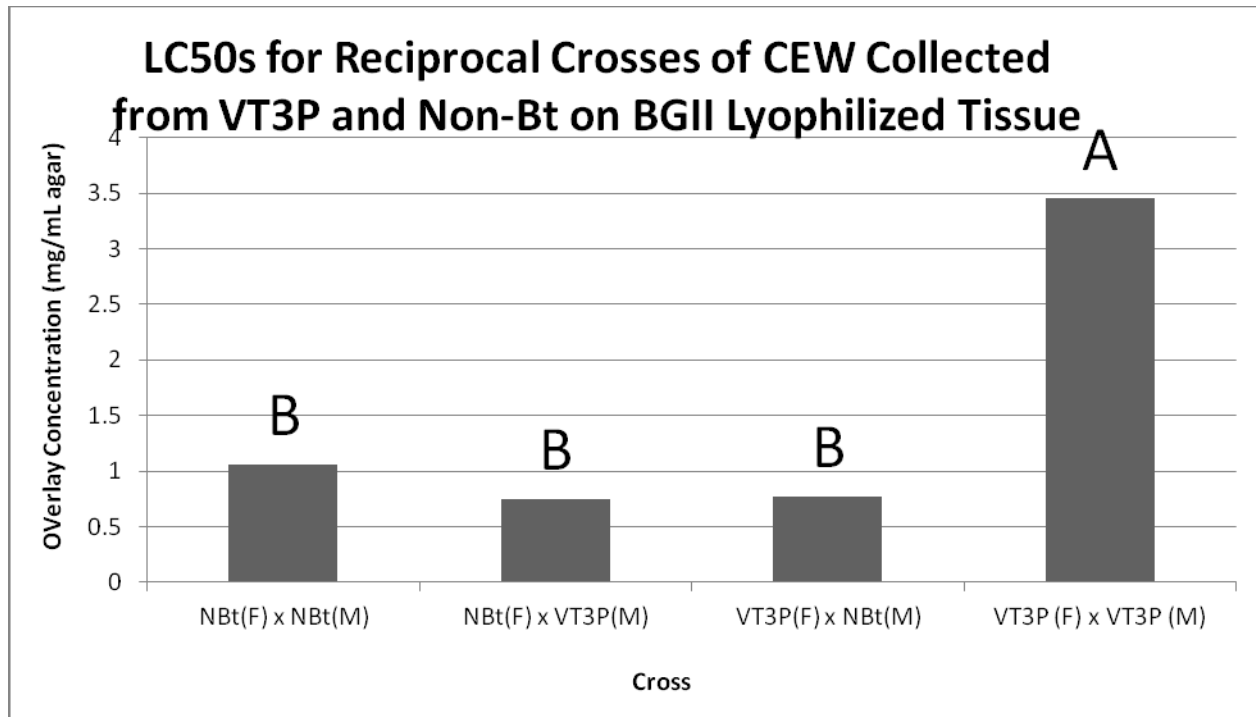


Figure 3. LC50s for Progeny Resulting from VT3 PRO and Non-Bt Field Corn on Bollgard II cotton in 2011.

Discussion

Results from the field corn survey are not indicative of larval densities infesting field corn from the previous two years (data not presented here). Furthermore, sample data taken on 24 July displaying high bollworm densities represents a small portion of the corn acreage in Mississippi because these fields were planted after the optimum planting period for corn. Overall, the data suggests field corn contributed very little to bollworm populations infesting cotton for the 2012 growing season which has not been true in previous years.

Assay data were similar for both years. The back cross in which both parents were reared on VT3 PRO was consistently higher in both years which indicate progeny from individuals developing on VT3 PRO corn are more virulent on Bollgard II cotton. Elevated LC50s were observed on larvae from moths completing one generation in VT3 PRO corn and because of this, the propensity for this phenomenon to be seen in successive generations completing development in transgenic corn should be high.

References

- Adamczyk, Jr., J.J., D. D. Hardee, L.C. Adams, and D.V. Sumerford. 2001. Correlating differences in larval survival and development of bollworm (Lepidoptera: Noctuidae) and fall armyworm (Lepidoptera: Noctuidae) to differential expression of Cry 1A(c) δ -endotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. J. Econ. Entomol. 94: 284-290.
- Gore, J., B.R. Leonard, and H. Jones. 2003. Influence of agronomic hosts on the susceptibility of *Helicoverpa zea* (Boddie) (Lepidoptera:Noctuidae) to genetically engineered and non-engineered cottons. Environ. Entomol. 32: 103-110.
- Greenplate, J.T., G.P. Head, S.R. Penn, and V.T. Kabuye. 1998. Factors potentially influencing the survival of *Helicoverpa zea* on bollgard cotton. In: Proceedings of the Beltwide Cotton Conferences. National Cotton Council, Memphis, TN. pp. 1030-1033.

Head, G., T. Dennehy. 2010. Insect resistance management for transgenic Bt cotton. *Biotechnology in Agriculture and Forestry*. 65: 113-125.

Isely, D. 1926. Protecting cotton from injury by the bollworm. *Arkansas Ext. Bull.* No. 218.

Isely, D. 1942. Insect problems resulting from changes in agriculture in Arkansas. *J. Econ. Entomol.* 35: 473-477.

Jackson, R.E., J.R. Bradley, J. Van Duyn, B.R. Leonard, K.C. Allen, R. Luttrell, J. Ruberson, J. Adamczyk, J. Gore, D.D. Hardee, R. Voth, S. Sivasupramaniam, J.W. Mullins, and G.Head. 2008. Regional assessment of *Helicoverpa zea* populations on cotton and non-cotton crop hosts. *Entomologia Experimentalis et Applicita*. 126: 89-106.

Kogan, M., C.G. Helm, J. Kogan, and E. Brewer. 1989. Distribution and economic importance of *Heliothis virescens* and *Heliothis zea* in North, Central and South America and of their natural enemies and host plants. *Proc. Bio. Cont. of Heliothis*. pp. 241-297.

Lincoln, C. 1972. Seasonal abundance. *In* Distribution, abundance and control of *Heliothis* species in cotton and other host plants. *USDA South. Coop. Ser. Bull.* 169: 2-7.