

Evaluation of Attribute II Bt Sweet Corn Resistance and Reduced-Risk Insecticide Applications for Control of Corn Earworm

R. Weinzierl, R. Estes, N. Tinsley, M. Keshlaf

Abstract—The corn earworm, *Helicoverpa zea* Boddie, is a serious pest of corn. Larval feeding in ear tips destroys kernels and allows growth of fungi and production of mycotoxins. Infested sweet corn is not marketable. Development of improved transgenic hybrids expressing insecticidal toxins from *Bacillus thuringiensis* (Bt) may limit or prevent crop losses. The effectiveness of Attribute® II Bt resistance and applications of Voliam Xpress insecticide were evaluated for effectiveness in controlling corn earworm in plots near Urbana, IL, USA, in 2013. Where no insecticides were applied, ear infestations and kernel damage in Attribute® II ‘Protector’ plots were consistently lower (near zero) than in plots of the non-Bt isolate ‘Garrison.’ Multiple applications of Voliam Xpress significantly reduced the number of corn earworm larvae and kernel damage in the Garrison plots, but infestations and damage in these plots were greater than in Protector plots that did not receive insecticide applications. Our results indicate that Attribute® II Bt resistance is more effective than multiple applications of an insecticide for preventing losses caused by corn earworm in sweet corn.

Keywords—*Bacillus thuringiensis*, *Helicoverpa zea*, insect pest management, transgenic sweet corn.

I. INTRODUCTION

SWEET corn, *Zea mays* L., is attacked by several insect pests that can cause significant losses. European corn borer, *Ostrinia nubilalis* (Hübner), fall armyworm, *Spodoptera frugiperda*, and corn earworm, *Helicoverpa zea* Boddie, are among the most damaging species in most of North America [1]. Corn earworm (CEW) adult females (moths) prefer to oviposit on the silks of sweet corn, and larvae move from the silks into the tips of ears where they feed on developing kernels [2]. Control of CEW is a major concern for growers of sweet corn in the Midwestern United States, and control strategies include early planting to avoid heaviest moth flights, application of insecticides from anthesis until harvest [3], biological control [4], and transgenic Bt sweet corn [5], [6].

Multiple genes that regulate the production of Bt toxins have been transferred into corn to provide insect resistance [7]. Generally, Bt plants have low toxicity to natural enemies [8], [9] and provide varying levels of control of Lepidopteran pests [6], [10], [11]. Transgenic sweet corn hybrids expressing the insecticidal protein Cry1Ab from *Bacillus thuringiensis* (Bt) var. *kurstaki* were the first to be used to limit losses to

CEW [11]. Genetic modification events such as Bt11 (Novartis Seeds) and MON810 (Monsanto Co.) resulted in production of Bt endotoxins in vegetative and reproductive structures throughout the season [10], [12], [13]. However, because plants are heterozygous for Bt toxin production (with the Bt production trait dominant), 25 percent of kernels (F_1 generation) in Bt sweet corn fields planted from seed with one genetic modification do not contain Bt toxins [5]. This allows low levels of CEW survival and crop contamination.

Because of incomplete control and concerns about the potential for corn earworm to develop virulence to Bt technology in transgenic corn, new hybrids containing a second gene for toxin production have been developed. The Attribute® II trait pyramid (Syngenta) contains genes that code production of vegetative insecticidal proteins (VIPs) and Cry proteins derived from *Bacillus thuringiensis*. VIPs differ from Cry proteins and bind to different receptors within an insect’s mid-gut membrane [5]. Expressing both VIP and Cry proteins reduces the risk of insect resistance and increases the portion of kernels that produce at least one Bt toxin to 94 percent [5]. Our objectives were to evaluate the efficacy of an Attribute® II transgenic Bt sweet corn hybrid and multiple applications of a reduced-risk insecticide to limit corn earworm infestations and damage in sweet corn.

II. METHODOLOGY

Evaluations were conducted at the Integrated Pest Management Research Farm at the University of Illinois near Urbana, Illinois. Plots were planted on July 1, 2013 and arranged in a randomized complete block design with four replications. Plots were planted with a 4-row SRES planter set for a population of 24,600 seeds/acre. Treatments included two sweet corn hybrids: (1) ‘Garrison,’ a yellow sugar-enhanced type for fresh market and (2) its Bt transgenic isolate, Attribute® II ‘Protector.’ For each hybrid, plots were treated (or not treated) with the insecticide Voliam Xpress (chlorantraniliprole + lambda-cyhalothrin) as listed in Table I. Plots consisted of 4 rows, 30-feet long. In insecticide-treated plots, the interior 2 rows were sprayed with 6 fl oz/acre of Voliam Xpress (in 20 gallons of water/acre) using a backpack sprayer operating at 40 psi. Nozzles (TTJ60-1102VP) were spaced at 20 inches on a 10-foot boom. Insecticide applications started on 23 August, one day after silk emergence began. Insecticide-treated Garrison plots received five applications at 3- to 4-day intervals (Table I). This treatment schedule is recommended where CEW moth flight is

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ongoing between first silk and harvest. Insecticide-treated Protector plots received two or three applications to determine if a reduced spray program would provide adequate control.

TABLE I
INSECTICIDE (CHLORANTRANILIPROLE + LAMBDA-CYHALOTHRIN)
APPLICATIONS FOR CONTROL OF CORN EARWORM ON SWEET CORN, URBANA,
IL, 2013. (GARRISON IS A NON-BT HYBRID; AND PROTECTOR IS ITS
ATTRIBUTE II BT-ENHANCED ISOLINE)

Hybrid	Treatment	Insecticide Application dates
Garrison	Untreated	None
Garrison	Full spray program	23, 27, 31 August, 4, 7 September
Protector	Untreated	None
Protector	Minimum sprays	23, 27 August
Protector	Minimum sprays	23, 31 August, 7 September

A. Magnitude of CEW Flight from Anthesis to Harvest

To monitor populations of adult CEW, two Hartstack wire cone traps [1] baited with 'Zealure' pheromone lures were established near the experimental plots. We collected and counted corn earworm male moths captured in these traps from 16 August to 8 September.

B. Ear Infestations and Damage

At maturation for fresh-market harvest, 25 ears were randomly hand-harvested from the center two rows of each plot on 11 September. Ears were examined for external damage, then husked to assess kernel damage and infestations. Assessments included counting the number of kernels damaged by insect feeding and categorizing CEW larvae as small (i.e., instar 1 and 2), medium (i.e., instar 3 and 4), and large (i.e., instar 5 and 6) in each ear [5], [14].

C. Statistical Analyses

Observations of damage (number of kernels) and insects were counts (number per 25 ears for each treatment in each replication) and consequently were transformed [$\log_{10}(x+1)$] to reduce the heterogeneity of variances before analysis by ANOVA (Microsoft Excel 2010). Where ANOVA indicated significant differences ($P \leq 0.05$) among treatments, Fisher's Protected LSD was used for mean separation. Least significant differences were calculated for and applied to the transformed data to determine differences in means among treatments, then those differences were used to describe the raw means presented in Table II.

III. RESULTS AND DISCUSSION

Corn earworm moth counts for the period from first silk through harvest are summarized in Fig. 1. Moth flight during this period was much lighter than average in late August and early September at this location; flights most years exceed 100 moths per night at this time (Weinzierl, unpublished data). However, most adjacent corn fields (dent corn grown for grain) had silked before the experimental plots, so our late-planted plots were the most attractive host plants in the area. Moth flight was adequate to result in severe infestation of the untreated Garrison plots.

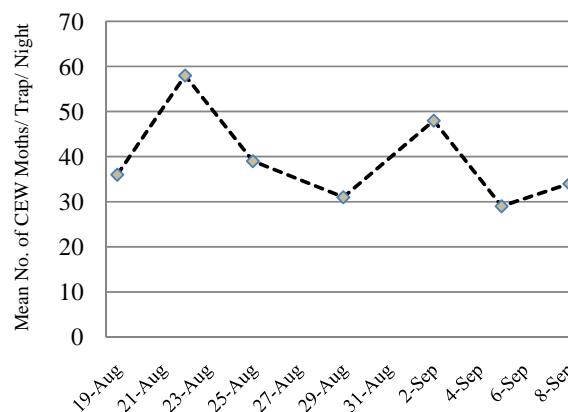


Fig. 1 Mean number of corn earworm, *Helicoverpa zea*, adults (moths/trap/night) in two of Hartstack traps, Urbana, IL (16 August to 8 September 2013)

Insect infestations and damage are summarized in Tables II and III. European corn borer and fall armyworm were very rare in the untreated check; damage was therefore attributed to corn earworm.

TABLE II
SIZE DISTRIBUTION AND TOTAL NUMBER OF CORN EARWORM LARVAE IN
SWEET CORN EARS, URBANA, IL, 2013

Hybrid	Mean number of CEW larvae/100 Ears		
	Small	Medium+ Large	Total CEW larvae
Garrison Untreated	7.0 ^a	67.0 ^a	74.0 ^a
Garrison (All Sprays)	13.0 ^a	17.0 ^b	30.0 ^b
Protector Untreated	1.0 ^b	1.0 ^c	2.0 ^c
Protector (2-Sprays)	0.0 ^b	0.0 ^c	0.0 ^c
Protector (3-Sprays)	0.0 ^b	0.0 ^c	0.0 ^c

*Means in the same column followed by the same letter do not differ significantly at $P = 0.05$

The abundance of CEW larvae in the untreated Garrison (non-Bt) plots suggests that larvae also hatched on silks of the Protector Bt plots and failed to survive after feeding on silks or kernels. The size (age) range of larvae and the consistently moderate captures of moths in traps indicate that larvae were entering ears throughout the period from first silk to harvest. The near-total absence of larvae in ear samples from the Protector Bt plots suggests that Bt toxins were present at sufficient levels to kill larvae from first silk to harvest. Although multiple insecticide applications reduced significantly ($P < 0.001$) larval infestations in the treated Garrison plots by nearly 60 percent in comparison with infestations in the untreated Garrison plots, 30 percent of the ears from the treated plots were infested. No larvae were found in samples from the Protector plots treated two or three times.

Nearly 90 percent of the ears from the untreated 'Garrison' plots were insect-damaged (Table III). Conversely, only 2% of the ears from the untreated Protector plots were damaged (Table III). In 100 ears from the untreated Protector plot, only 10 kernels were damaged (in contrast to 1,290 damaged

kernels in 100 ears from the untreated Garrison plot.

Multiple insecticide applications reduced significantly ($P < 0.001$) the number of damaged ears and kernels in the Garrison plots, but as with assessments of larval infestations, insecticide applications did not provide adequate protection; 59 percent of the harvested ears were damaged (1 or more kernels per ear), and an average of 4.8 kernels per ear were partially or entirely consumed. No ears or kernels were damaged in samples from the insecticide-treated Protector plots, but this result did not differ significantly from the damage level in the untreated Protector plots (2 ears per 100 and 0.1 kernel per ear). Overall, the untreated Protector plots were nearly undamaged and insect-free, and minimal spray programs resulted in no damage or infestations.

TABLE III
MEAN NUMBER OF UNDAMAGED EARS PER 25 EARS AND DAMAGED KERNELS PER EAR IN SWEET CORN AT HARVEST, URBANA, IL, 2013

Hybrid	Undamaged Ears /100 Ears	Damaged Kernels/ Ear
Garrison Untreated	22.25 ^a	12.9 ^a
Garrison (All Sprays)	14.75 ^b	4.8 ^b
Protector Untreated	24.5 ^c	0.1 ^c
Protector (2-Sprays)	25 ^c	0.0 ^c
Protector (3-Sprays)	25 ^c	0.0 ^c

*Means in the same column followed by the same letter do not differ significantly at $P = 0.05$

Our data demonstrate that this Attribute[®] II hybrid, with genes that code for production of Vip3A and Cry1Ab toxins, suffers little or no damage if grown with or without insecticide application when moderate levels of corn earworm pressure exist.

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