CORN EARWORM EMERGENCE FROM VIP3A SEED BLEND VS BLOCK REFUGE EARS

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Abstract

The three-year field study was conducted to compare the emergence of corn earworm, *Helicoverpa zea* between seed blend and lock refuge systems. The blocks of 95:5% seed blend of Bt (P1401VYHR) and non-Bt (P1498R) corn were planted in 2014 and 2015. In 2019, the blocks of 90:10% seed blend of Bt (DKC66-29) and non-Bt (DKC66-94) corn were planted. The emergence of corn earworm from block and seed blend refuge ears was monitored using aluminum screen cages. In addition, data were gathered on the presence of Bt toxins in tip kernels of non-Bt ears from seed blend refuge plots. Significantly fewer numbers of corn earworms were recovered from the seed blend refuge compared to the block refuge in two out of three years of the study. Also, the amount of corn earworm injury was significantly lower in non-Bt ears from seed blend than the block refuge ears. The percentage of kernels from non-Bt ears of seed blend refuge with expression of at least one of the Bt toxins ranged from 52% to 76%. Our results agree with past studies that pollen flow from Bt plants to refuge plants is high, resulting in a mosaic of kernels expressing single or multiple toxins. Such a mosaic of non-Bt and single and multiple toxin kernels can reduce the number of homozygous susceptible larvae produced and favor survival of heterozygotes and selection for resistance.

Introduction

This The high-dose refuge strategy is a primary IRM tactic for transgenic corn expressing Bt insecticidal toxins. A structured refuge consists of a block of non-Bt corn planted adjacent to or near the Bt corn. Whereas, seed blend refuge involves mixing of 5% or 10% non-Bt seed in a bag of Bt corn (Tabashnik et al. 2008). In theory, refuge provides an area for susceptible insects to develop without selection for resistance to Bt toxins, and thus are available to interbreed with potentially homozygous resistant individuals that may emerge from the Bt plants. The seed blend system provides farmers with a convenient and easier compliance solution to meet federal regulations (Matten et al. 2012). However, there is concern about the Bt pollen flow between Bt and non-Bt plants in seed blend plantings. Pollen flow from Bt plants to refuges can result in toxin expression in the kernels of refuge plants (Yang et al. 2014). Such Bt contamination may affect target pests that feed on corn ears such as corn earworm, *Helicoverpa zea*. Here, we present the results from field experiments to compare the emergence of corn earworm from seed blend and block refuge systems. We also provide information from gene-check assays as evidence of Bt toxin expression in ears of refuge plants in the seed blend system.

Materials and Methods

The study was conducted at the Texas A&M AgriLife Research and Extension Center in Lubbock, TX. The planting occurred on May 2, April 23, and June 4 in 2014, 2015, and 2019, respectively. In 2014 and 2015, 95:5% seed blend of Leptra (P1401VYHR) and P1498R non-Bt corn was planted. In 2019, 90:10% seed blend of Trecepta (DKC66-29) and DKC66-94 non-Bt was used.

Non-Bt seed blend plants (2014: 125 plants/treatment, 2015: 90 plants/treatment, and 2019: 40 plants/treatment) were identified by using signs of insect feeding and verified as non-Bt with EnviroLogix QuickStix that detect Vip3a. Experimental ears from both seed blend and bock refuge plots were covered with aluminum window screen cages (15 X 6 inches) during the milk stage. One day prior to cage installation, ears from both treatments that were not destined for the experiment were dissected and larvae counted to assure similar initial infestation levels between treatments. The cages were left undisturbed until harvest. At crop maturity, plants with screen cages were cut below and above the ear and the caged ears with attached stalks were taken to the laboratory. Ears were dissected and the number of larvae, pupae, and adult stages of corn earworm were recorded.

After insect data were taken, 10 tip kernels from randomly selected non-Bt ears from seed blend refuge plots were assayed for the presence of Bt toxins using EnviroLogix QuickStix that detect all possible Bt toxins.

Chi-square analysis was used to compare insect emergence between block and seed blend refuge ears. Similarly, Chi-square was used to compare treatments for % ears with corn earworm damage. A T-test was used to compare treatment means for amount of earworm damage, and results were confirmed with a Wilcoxon Signed Rank test.

Results and Discussion

The total number of corn earworms emerged from block refuge and seed blend refuge did not vary significantly in 2014 ($\chi^2 = 0.07$, $P = 0.7913$) (Fig. 1). In 2015 ($\chi^2 = 17.82$, $P = 0.000024$) and 2019 ($\chi^2 = 6.37$, $P = 0.0116$), however, significantly fewer numbers of corn earworms were recovered from the seed blend refuge compared to the block refuge.

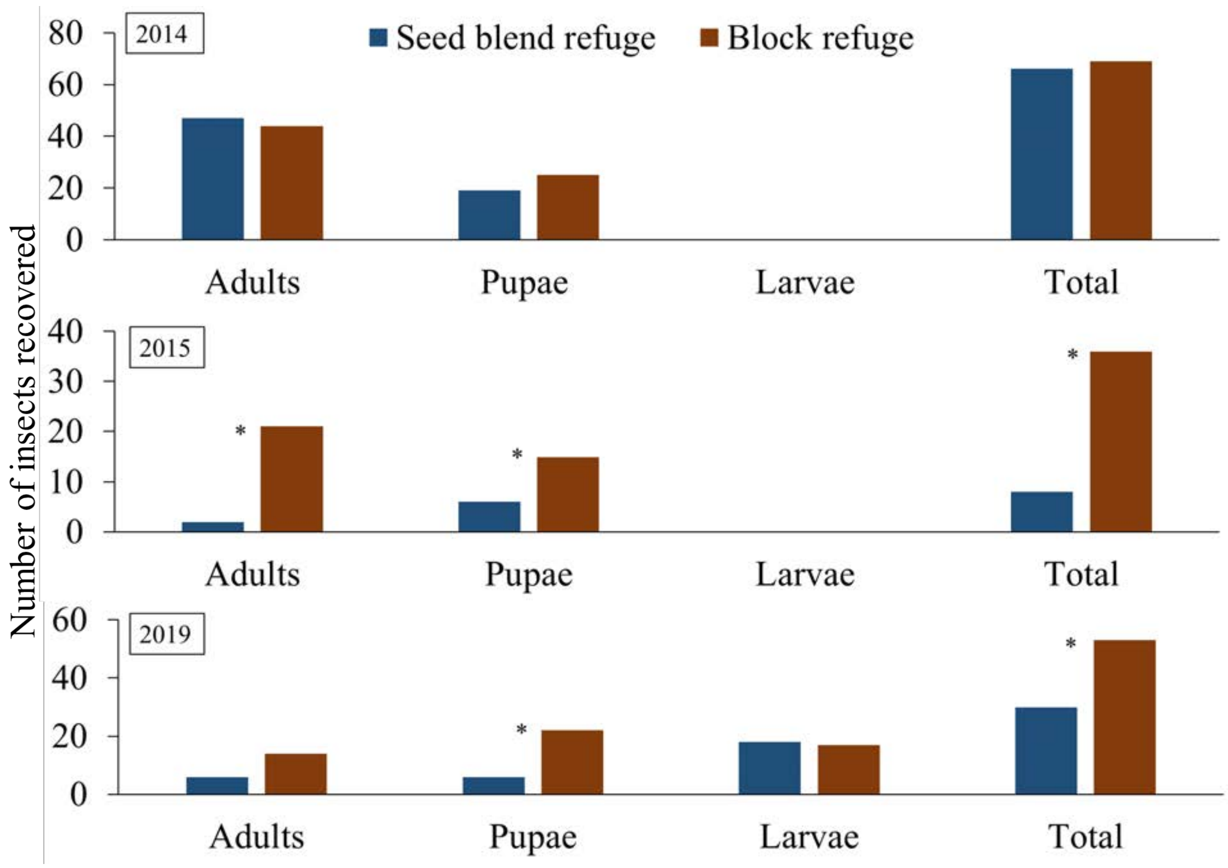

Fig. 1. Number of corn earworms recovered from seed blend and block refuge ears. *indicates significant difference between means at alpha = 0.05.

Percentage of ears with corn earworm damage was significantly lower in seed blend refuge compared to the block refuge in 2015 (Fig. 2A) ($\chi^2 = 18.97$, $P = 0.000013$). In 2019, however, no significant difference was observed in percent damaged ears between two treatments ($\chi^2 = 0.86$, $P = 0.3537$). The amount of corn earworm injury/feeding was significantly higher in block refuge ears than the non-Bt ears from seed blend refuge ($P < 0.0001$) (Fig. 2B).

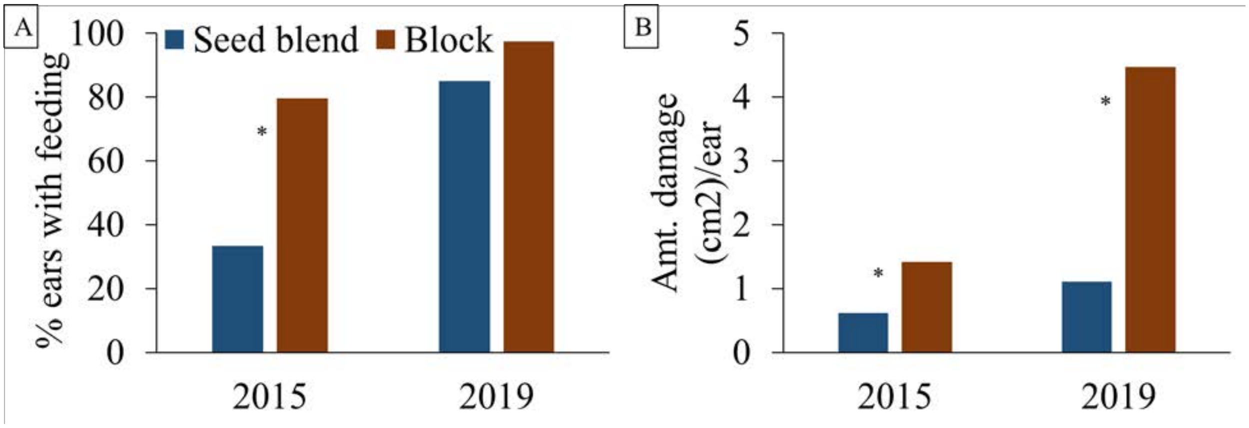

Fig. 2. Percentage of damaged ears (A) and amount of kernel feeding on damaged ears (B) in seed blend vs block refuge ears. *indicates significant difference between means at alpha = 0.05.

The percentage of kernels from non-Bt ears of seed blend refuge with expression of at least one of the Bt toxins was 74%, 52%, and 76% in 2014, 2015, and 2019, respectively (Table 1 and Fig. 3).

Table 1. Bt toxin/s in kernels of non-Bt ears in seed blend refuge, 2014-2015

		NO TOXIN	**Cry1Ab**	**Cry1F**	**Vip3a**	**Cry1Ab/ Cry1F**	**Cry1Ab/ Vip3a**	**Cry1F/ Vip3a**	**ALL**
2014		26%	8%	10%	11%	15%	11%	7%	12%
		NONE	One Toxin Present			Two Toxins Present			ALL
	Total	**26%**	**29%**			**33%**			**12%**
2015		48%	5%	10%	10%	4%	5%	13%	5%
		NONE	One Toxin Present			Two Toxins Present			ALL
	Total	**48%**	**25%**			**22%**			**5%**
Average		**38%**	**27%**			**28%**			**8%**

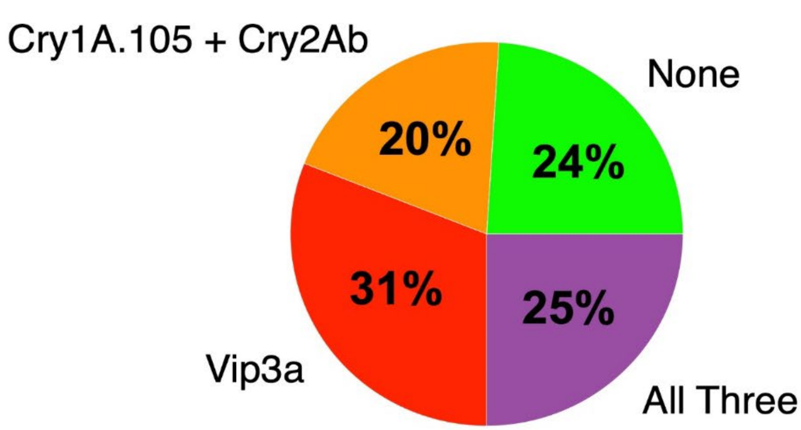

Figure 3. Bt toxin/s in kernels of non-Bt ears in seed blend refuge, 2019.

Our results agree with studies by Dively et al. (2019) and Yang et al. (2014) that pollen flow from Bt plants to refuge plants is high, resulting in a mosaic of kernels expressing single or multiple toxins. Such a mosaic of non-Bt and single and multiple toxin kernels can reduce the number of homozygous susceptible larvae produced and favor survival of heterozygotes and selection for resistance.

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