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Title: Reducing fall armyworm and western bean cutworm damage in strip refuges through toxic pollen from nearby Bt corn

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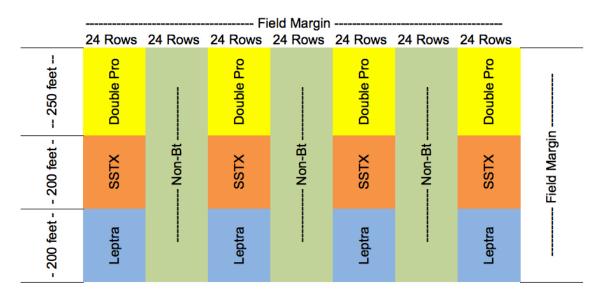
Executive Summary

This year's research showed that strip refuges adjacent to three types of Bt corn have fewer fall armyworm larvae than more distant strip refuge rows and that there is a strong trend toward less side entry and shank damage in refuge rows adjacent to Bt corn. Corn earworm numbers were not affected by proximity to Bt corn. These data, when combined with our 2013 data, are sufficient to recommend strip refuges when fall armyworm populations are moderate or light. Strip refuges of 4-6 rows should be a viable option for corn that is planted in a timely manner, but we do not recommend them for late planted corn where fall armyworm populations are often higher in number. We were unable to establish an experiment for western bean cutworm in the northern Panhandle.

Methods

Three types of Bt corn were planted on the north side of a pivot-irrigated field near Muleshoe, Texas on May 30th, 2014. Hybrids were DKC63-55 (Genuity Double Pro), DKC61-16 RIB (SmartStax 95:5 Refuge in a Bag), P1401VYHR (Pioneer Leptra), and DKC62-95 (RR2) non-Bt. 650 feet x 24 rows of non-Bt x 3 replications were surrounded on both sides by Bt corn as illustrated in Figure 1. This arrangement, while not random, allowed maximum confidence that pollen moving into the non-Bt strip was from the same Bt hybrid. The downside is that we could not compare insect numbers in the non-Bt across hybrids.

Figure 1. Arrangement of hybrids in the Muleshoe strip refuge protection trial.



Double Pro: DKC63-55 (GENDGVT2P)

SmartStax: DKC61-16 RIB Leptra: P1401VYHR Non-Bt: DKC62-95 (RR2)

The first sampling period was 8/13 - 8/14, 2014. In each block of Bt corn the 12th row of the 24 row block was sampled 90 feet from the start of the block. The test was designed to determine the number of insects and ear damage in refuge rows adjacent to Bt corn and in rows farther away from Bt corn. In the 24-row non-Bt blocks, refuge rows 1, 2, 4, 12, 20, 23 and 24 were sampled. The data from row 1 and 24 were pooled because each of these rows was adjacent to Bt corn. Similarly the data from non-Bt rows 2 and 23 were pooled to represent a refuge row two rows away from Bt corn. The data from non-Bt rows 4 and 20 were also pooled to represent rows 4 rows away from Bt. Rows 12 and 13 of the non-Bt strip were equidistant between the two Bt pollen sources and 20 ears were sampled from each of row 12 and 13 and pooled. All sampling was done by starting near the middle of the block and sampling every 5th ear. Skipping 4 ears helped eliminate "hotspots" caused by the presence of a fall armyworm egg mass. The number of small ($< \frac{1}{2}$ "), medium (1/2" - 1") and large (> 1") fall armyworm and corn earworm larvae were recorded. Preliminary analysis of the data showed clear reduction in fall armyworm numbers in refuge rows adjacent to Double Pro and Leptra, but inconsistent trends in SmarStax. We therefore re-sampled the SmartStax refuge rows on 8/22. On 9/2 ten ears in each Bt block and refuge rows 1, 2, 4 and 12 on the east side of the Bt block were examined for shank damage and ear side damage (larval entry).

Results and Discussion

Corn Earworm:

We found that none of the three types of Bt corn protects adjacent strip refuges from corn earworm. The number of larvae recovered in adjacent refuge rows 1 and 2 was not different from the number recovered in the distant refuge row 12 (Table 1). Fortunately, corn earworm is not a major source of yield loss.

Table 1. Mean number of corn earworm larvae per ear for each Bt type and adjacent

refuge rows. Milk stage, 8/13 – 8/14.

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Row	No. of	Double Pro ¹	Smartstax ²	Leptra ³	
	ears				
Purestand	60	0.30 a (0.30)	0.53 a (0.22)	0.00 a (0.00)	
Refuge Row 1	120	1.36 b (0.08)	1.22 b (0.13)	1.41 b (0.02)	
Refuge Row 2	120	1.36 b (0.30)	1.31 b (0.09)	1.28 b (0.14)	
Refuge Row 4	120	1.24 b (0.18)	1.35 b (0.16)	1.45 b (0.22)	
Refuge Row 12+13	120	1.09 b (0.06)	1.23 b (0.11)	1.28 b (0.10)	

 $^{^{1}}$ ANOVA Treatment Pr > F = .0042. Replication Pr > F = .6035

Fall Armyworm

Both Double Pro and Leptra provided significant protection to refuge rows 1, 2 and 4 as shown by fewer fall armyworm larvae recovered from these rows than refuge row 12 that received less Bt pollen from the Bt block. SmartStax seemed to provide similar protection but this was not quite statistically significant (P > F = 0.0743 on 8/13 - 8/14 and 0.0764 on 8/22) (Tables 2 and 3). In terms of preventing side entry and shank damage, there was s strong numerical trend for refuge rows closest to Bt block to have less damage. Double Pro and SmartStax showed statistically significant protection (refuge row 1 and/or 2 compared to refuge row 12), but all three types of Bt provided numerically better protection to refuge rows closest to the Bt blocks (Table 4). Larger sample sizes would probably have shown statistical significance in Leptra.

Table 2. Mean number of fall armyworm larvae per ear for each Bt type and adjacent refuge rows. Milk stage, 8/13 – 8/14. Figure 2 presents these data in graphical form.

Row	No. of	Double Pro ¹	Smartstax ²	Leptra ³
	ears	(std. dev)		
Purestand	60	0.33 a (0.03)	0.20 a (0.20)	0.00 a (0.00)
Refuge Row 1	120	0.48 ab (0.03)	0.53 b (0.11)	0.35 b (0.05)
Refuge Row 2	120	0.46 ab (0.05)	0.53 b (0.09)	0.38 b (0.50)
Refuge Row 4	120	0.59 b (0.11)	0.41 ab (0.08)	0.37 b (0.09)
Refuge Row 12	120	0.77 c (0.10)	0.53 b (0.11)	0.68 c (0.07)

 $^{^{2}}$ ANOVA Treatment Pr > F = .0018. Replication Pr > F = .9724

 $^{^{3}}$ ANOVA Treatment Pr > F < .0001. Replication Pr > F = .7215

Table 3. Corn earworm and fall larvae in refuge rows adjacent to Smartstax, 8/22/14. Purestand not sampled. Supplemental data to examine the lack of response the prior week.

Row	No. of	Corn earworm ¹	Fall armyworm ²
	ears	(std. dev)	(std. dev)
Refuge Row 1	60	0.78 (0.11)	0.43 (0.15)
Refuge Row 2	60	0.75 (0.06)	0.63 (0.11)
Refuge Row 4	60	0.75 (0.06)	0.58 (0.17)
Refuge Row 12	60	0.73 (0.12)	0.85 (0.09)

 $^{^{1}}$ ANOVA Treatment Pr > F = .3925. Replication Pr > F = .1066

Table 4. Mean proportion of ears with side entry damage, shank damage or both in solid Bt plantings and refuge rows, 9 August. Figure 3 presents these data in graphical form.

Row	No. of	Double Pro ¹	Smartstax ² (std.	Leptra ³ (std. dev)
	ears	(std. dev)	dev)	
Purestand	40	0.00 a (0.00)	0.03 a (0.04)	0.00 a (0.00)
Refuge Row 1	40	0.08 b (0.04)	0.15 b (0.02)	0.15 b (0.08)
Refuge Row 2	40	0.17 c (0.04)	0.15 b (0.03)	0.20 b (0.16)
Refuge Row 4	40	0.27 d (0.04)	0.27 b (0.07)	0.33 b (0.14)
Refuge Row 12	40	0.37 d (0.05)	0.37 b (0.11)	0.32 b (0.06)

ANOVA values and mean separations based on analysis of arcsine of the square root analysis of proportions. Means presented are arithmetic means.

 $^{^{1}}$ ANOVA Treatment Pr > F = .0016. Replication Pr > F = .4946

 $^{^{2}}$ ANOVA Treatment Pr > F = .0734. Replication Pr > F = .6171

 $^{^{3}}$ ANOVA Treatment Pr > F < .0001. Replication Pr > F = .8364

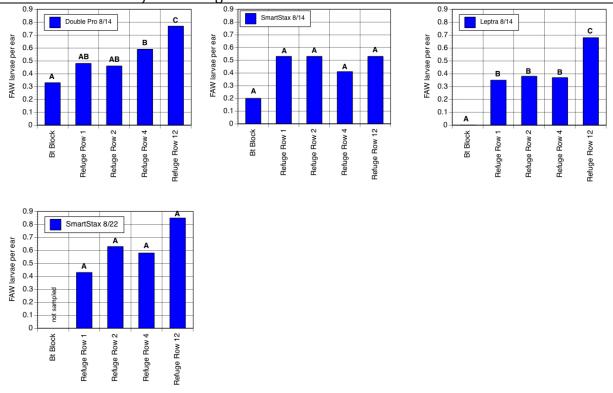
 $^{^{2}}$ ANOVA Treatment Pr > F = .0764. Replication Pr > F = .3185

 $^{^{1}}$ ANOVA Treatment Pr > F < .0001. Replication Pr > F = .2103

 $^{^{2}}$ ANOVA Treatment Pr > F = .0039. Replication Pr > F = .6670

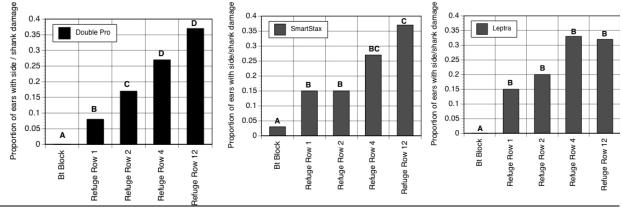
 $^{^{3}}$ ANOVA Treatment Pr > F < .0014. Replication Pr > F = .3841

Figure 2. Graphical representations of fall armyworm larvae per ear in three types of Bt corn and their adjacent refuge rows. Data are the same as in Table 2*.



^{*} SmartStax had no statistically significant differences on either date: P > F = 0.0743 on 8/13 - 8/14 and 0.0764 on 8/22.

Figure 3. Mean proportion of ears in three types of Bt corn and their adjacent refuge rows with side entry damage, shank damage or both. Data are taken from Table 4.



Two years of data have shown that 4 – 6 row strip refuges receive protection from fall armyworm (but not corn earworm) by virtue of Bt pollen flowing from adjacent Bt corn. This protection amounts to a 40 – 50% reduction in the number of caterpillars and comes from two factors; Bt pollen is toxic to small caterpillars and the kernels in non-Bt ears that result from pollination by Bt pollen are toxic or partially toxic. While this protection was not statistically evident for SmartStax in 2014, we think that SmartStax will provide protection equivalent to Double Pro. This is because SmartStax contains both of the toxins in Double Pro (Cry 1A.105 + Cry2Ab2) and one additional toxin (Cry1F). We believe the non-performance of SmartStax in refuge row protection in this experiment is a statistical anomaly.

We are confident that strip refuges have a fit in refuge compliance, but there are limits. We visited late planted strip refuge fields in Cochran County this year that had extremely high numbers of fall armyworm and corn earworm larvae per ear. Fall armyworms averaged 4 per ear and corn earworms averaged around 3. In this case the Double Pro strip refuges did not reduce the number of fall armyworms and it was apparent that strip refuge protection breaks down under very high infestation levels. We are therefore recommending that strip refuges only be considered for corn that is planted within the normal planting window.

Additionally, it will probably not be economical to spray a 20% strip refuge field, so growers who want the option to spray should plant block refuges. One final caveat is that there is still some single toxin Herculex corn (Cry1F) being grown and we do not think Herculex has the ability to protect adjacent strip refuge rows.

We have summarized our strip refuge recommendations in an article to be published in the Texas Corn Seed Book this month.

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