Ability of SmartStax RIB and Double Pro to protect adjacent strip refuges from corn earworm and fall armyworm, and a look at pollen movement from Bt blocks to adjacent strip refuges

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Summary

The purpose of this research was to determine whether pollen from adjacent Bt corn can protect corn ears in adjacent non-Bt rows. If so, then strip refuges become an option that allows growers to comply with refuge requirements while experiencing less loss from insects than would happen in a large block refuge. 12-row blocks of SmartStax 5% Refuge in a bag and Double Pro Bt corn were able to protect adjacent strip refuge rows from corn earworm (CEW) and fall armyworm (FAW) larvae to different degrees. Double Pro offered adjacent refuge rows no protection from either corn earworm or fall armyworm. SmartStax offered adjacent refuge rows significant protection from fall armyworm but not corn earworm.

1. Corn earworm: Neither type of Bt corn offered significant protection from corn earworm larvae in adjacent refuge rows 1, 4 or 12 (CEW Analysis 6). There was not a significant difference in the number of larvae recovered in refuge rows 1, 4, and 12 (CEW Analysis 5). Taken together, this suggests that neither type of Bt corn is able to protect adjacent strip refuge rows from CEW. However, within the solid blocks of Bt corn, both types of corn had fewer corn earworm larvae in ears than in the refuge rows, and this indicates that both technologies provide significant insect control in block plantings. The SmartStax Bt block had fewer total larvae than the Double Pro Bt block at the 0.07 level of probability (CEW Analysis 2), which is expected since SmartStax contains the toxins in Double Pro and additionally has the Cry1F toxin and should therefore be more effective than Double Pro. Larvae recovered from within the solid plantings were significantly smaller than those recovered from refuge rows (CEW Analyses 3 and 4).

2. Fall armyworm: Refuge rows adjacent to SmartStax had significantly fewer FAW larvae than those more distant from the block planting and those adjacent to Double Pro (FAW Analysis 1), which indicates that there is potential for strip refuges adjacent to SmartStax to receive significant protection. At dough stage the solid SmartStax block planting and refuge row 1 (closest to the SmartStax block) had the lowest numbers of FAW larvae, and the numbers of

larvae in refuge rows increased with distance from the solid planted block (FAW Analysis 2A). This trend increased in intensity by early dent stage such that there was a near linear increase in FAW numbers in refuge rows 1, 2, 4 and 12 with distance from the solid Bt block (FAW Analysis 2B). These data suggest that refuge rows 1, 2, and perhaps 4 can be protected by an adjacent SmartStax block and that 4 – 8 row strip refuges might be adequately protected in production fields when surrounded on both sides by SmartStax corn.

Unlike SmartStax, pollen from Double Pro corn offered refuge rows no significant protection from FAW (FAW Analysis 3).

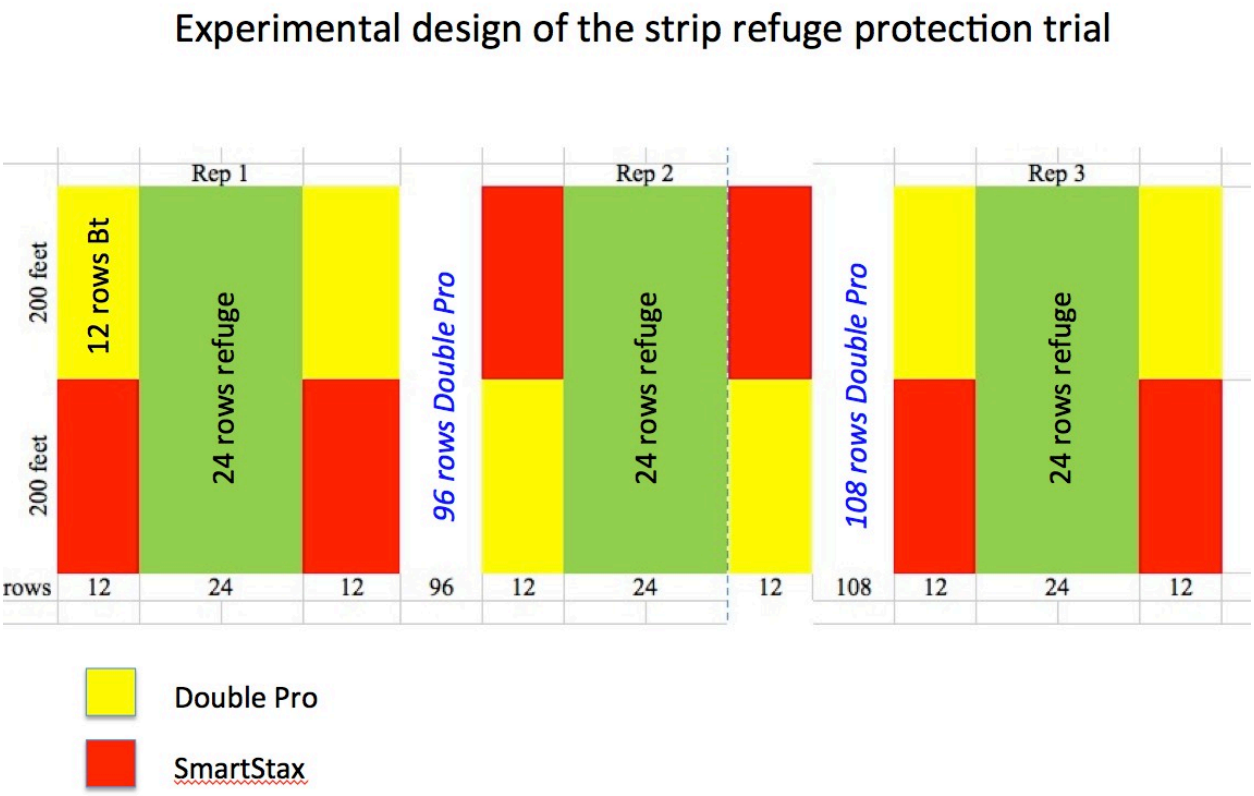
Bt pollen expression in adjacent refuge row non-Bt ears: The data clearly showed that ears in refuge rows closest to the SmartStax block had higher SmartStax toxin expression than ears from more distant refuge rows (Pollen Table 1). While not directly measured kernel by kernel, one can assume (and gene-check quick strip coloration suggests) that ears from refuge rows nearer the SmartStax block had a higher percentage of kernels expressing all of the toxins in SmartStax than ears farther from the refuge block. There was some segregation of SmartStax toxins such that not all of them were present in the top 1/3 of some of the refuge ears tested (Pollen Table 2). Similar trends for decreasing toxin expression with distance from the solid Bt planting and increasing segregation with distance were found for Double Pro (Pollen Table 3).

Toxin assays of individual kernels from SmartStax refuge row 2 revealed that 31.6% of them were positive for at least one toxin active against caterpillars (Pollen Table 4A). Of the 120 individual kernels tested, 5.8% were positive for Cry1F only, 10.8% were positive for Cry2Ab2, and 9.2% were positive for both toxins. Toxins were not detected in the remaining 74.2% of kernels in refuge row 2 (Pollen Table 5).

The presence of toxic kernels in adjacent refuge row non-Bt ears can partially explain the reduced number of FAW larvae in refuge rows closest to the solid Bt block plantings. It is also probable that the presence of the toxic pollen itself helped to kill some of the small caterpillars on refuge row ears. In summary, the results presented here indicate that 4-8 row strip refuges in SmartStax corn will very likely receive protection from fall armyworm larvae.

Methods

Plot arrangement: In 2013 near Muleshoe, Texas, 200-foot long strips of 12 rows of SmartStax RIB or Double Pro corn were planted in a center pivot irrigated field on 30-inch rows oriented north to south. The prevailing winds are usually from the west or southwest. Green refuge seed was observed in the bag of SSTX RIB.



The Bt block plantings were 12 rows wide x 200 feet long. There were 24 rows of non-Bt corn planted between twin blocks of Bt corn. The SmartStax corn was DKC 61-16 RIB (5%). The Double Pro was DKC 63-55 DGVT2P. The refuge corn was DKC 62-95 RR2.

Experimental plots were at the edge of a commercial corn field planted on 30-inch rows and irrigated by center pivot. Insecticides were not applied to this field.

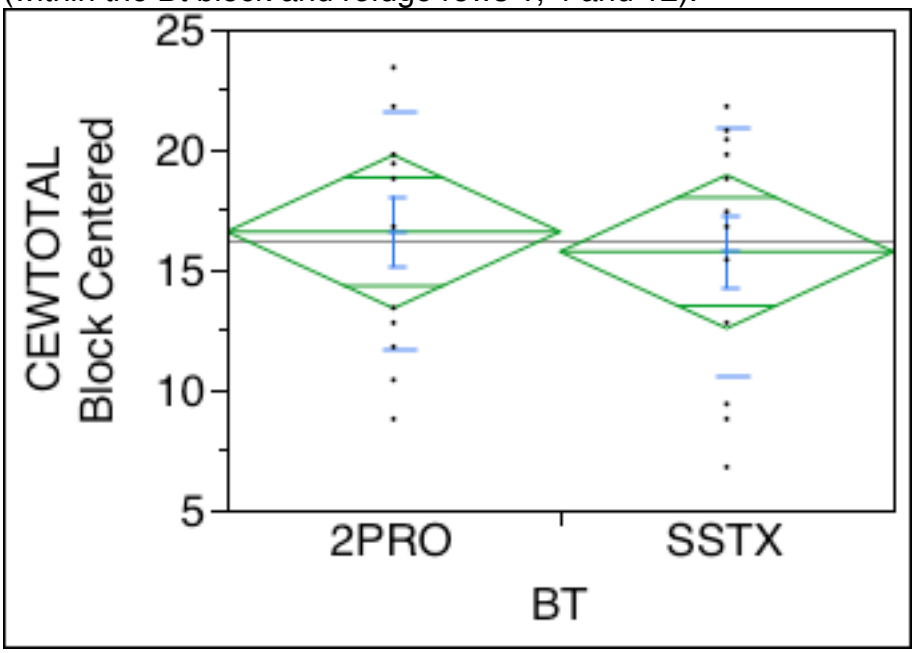
Insects were sampled at dough stage (19 – 20 August) and at early dent stage (6 September).

PART I. Corn Earworm

Ten consecutive ears per refuge row (or in row 6 of the 12-row pure Bt block) were harvested on 19 August at dough stage, removed to the laboratory and dissected. Larvae were scored as small (<¼ inch), medium (1/4 to ¾ inch) or large (> 3/4 inch). There were three replications.

CEW Analysis 1. Mean total number of CEW larvae recovered per 10 consecutive ears in all sampled rows by type of Bt corn. Data include recovery from the Bt block planting and refuge rows 1, 4 and 12. Finding: there were no significant differences in the number of larvae recovered from SmartStax or Double Pro.

Mean total CEW recovered per 10 ears by Bt type at dough stage, all rows included (within the Bt block and refuge rows 1, 4 and 12).



Analysis of Variance

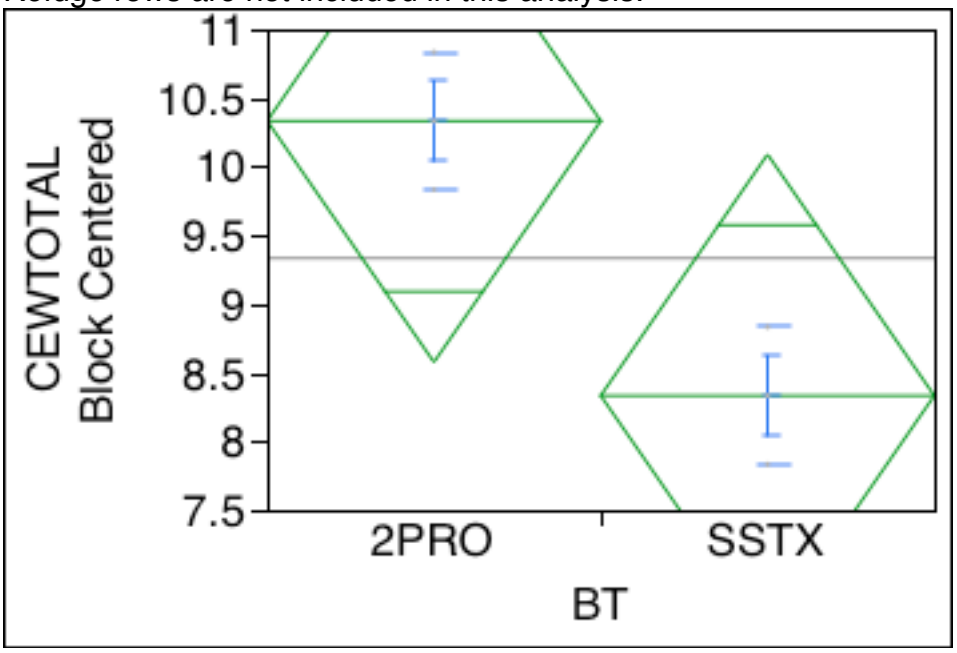
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
BT	1	4.16667	4.1667	0.1485	0.7040
REP	2	170.08333	85.0417	3.0313	0.0708
Error	20	561.08333	28.0542		
C. Total	23	735.33333			

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
2PRO	12	16.5833	4.95701	1.4310	13.434	19.733
SSTX	12	15.7500	5.14156	1.4842	12.483	19.017

CEW Analysis 2. Mean total number of CEW larvae recovered per 10 consecutive ears from row 6 in the Bt block planting (only). Refuge rows are not included in this analysis. Finding: At the 0.074 level of probability, SmarStax had fewer CEW larvae in the Bt block than were recovered from Double Pro.

Mean total number of CEW larvae recovered per ten ears in the Bt block planting (only). Refuge rows are not included in this analysis.



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
BT	1	6.000000	6.0000	12.0000	0.0742
REP	2	52.333333	26.1667	52.3333	0.0187*
Error	2	1.000000	0.5000		
C. Total	5	59.333333			

Replication Means

REP	Mean	Number
1	13.5000	2
2	7.5000	2
3	7.0000	2

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
2PRO	3	10.3333	0.500000	0.28868	9.0913	11.575
SSTX	3	8.3333	0.500000	0.28868	7.0913	9.575

Mean separation by t-Test
SmarStax vs. Double Pro Assuming unequal variances

Difference	-2.0000	t Ratio	-4.89898
Std Err Dif	0.4082	DF	4
Upper CL Dif	-0.8665	Prob > ltl	0.0080*
Lower CL Dif	-3.1335	Prob > t	0.9960
Confidence	0.95	Prob < t	0.0040*

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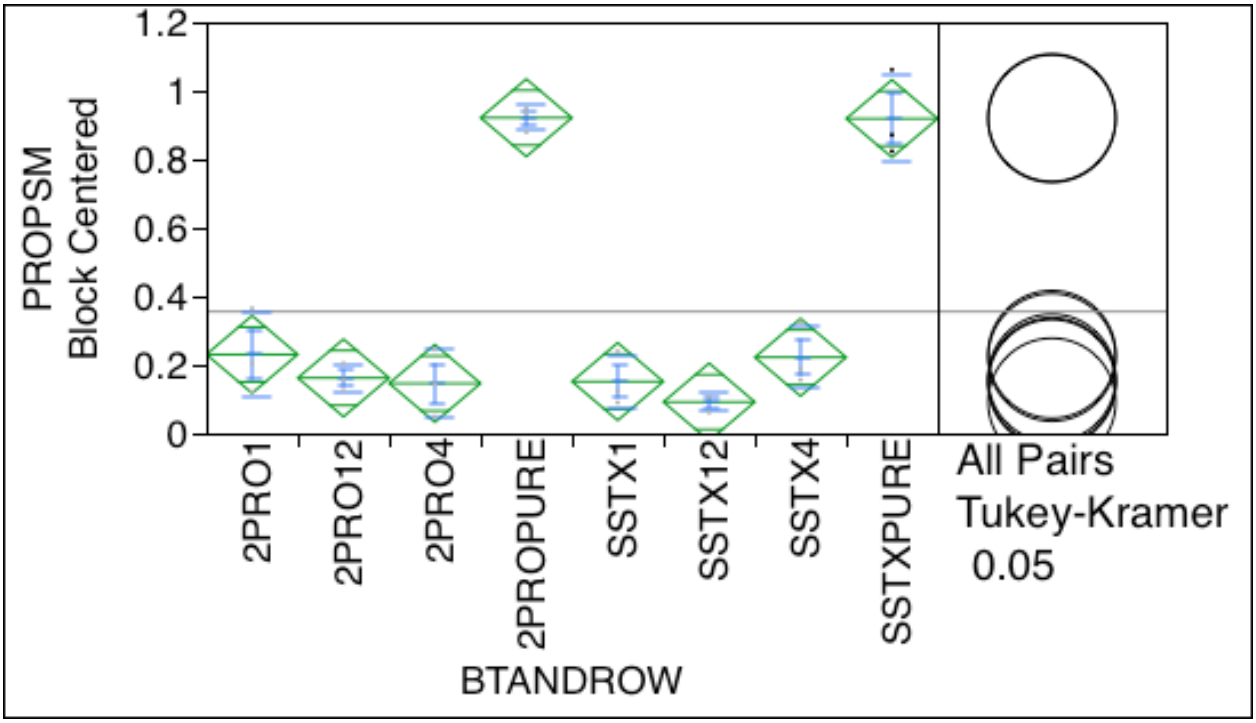
CEW Analysis 3. Proportion of small CEW larvae recovered by Bt type and refuge row. The data include sampling in the pure Bt block. Finding: A significantly higher proportion of larvae recovered from the pure Bt blocks were small. This indicates slower development in the pure Bt blocks than in any of the refuge rows for either Bt type.

Mean PROPORTION of CEW larvae that were small (< ¼ inch) by Bt type and refuge row. This analysis includes rows in the solid Bt block and refuge rows 1, 4 and 12. "2PROPURE" is Double Pro pure Bt block and SSTXPURE is the pure stand of SmartStax 5% refuge in a bag. Refuge row location is indicated as the last two digits in the row name.



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
BTANDROW	7	2.5897371	0.369962	44.3118	<.0001*
REP	2	0.0521030	0.026052	3.1203	0.0757
Error	14	0.1168871	0.008349		
C. Total	23	2.7587273			

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
2PRO1	3	0.230590	0.123692	0.07141	-0.0767	0.5379
2PRO12	3	0.161998	0.040196	0.02321	0.0621	0.2619
2PRO4	3	0.146151	0.097739	0.05643	-0.0966	0.3889
2PROPURE	3	0.921296	0.036902	0.02131	0.8296	1.0130
SSTX1	3	0.151389	0.078558	0.04536	-0.0438	0.3465
SSTX12	3	0.091847	0.026499	0.01530	0.0260	0.1577
SSTX4	3	0.222222	0.089155	0.05147	0.00075	0.4437
SSTXPURE	3	0.918803	0.125664	0.07255	0.6066	1.2310

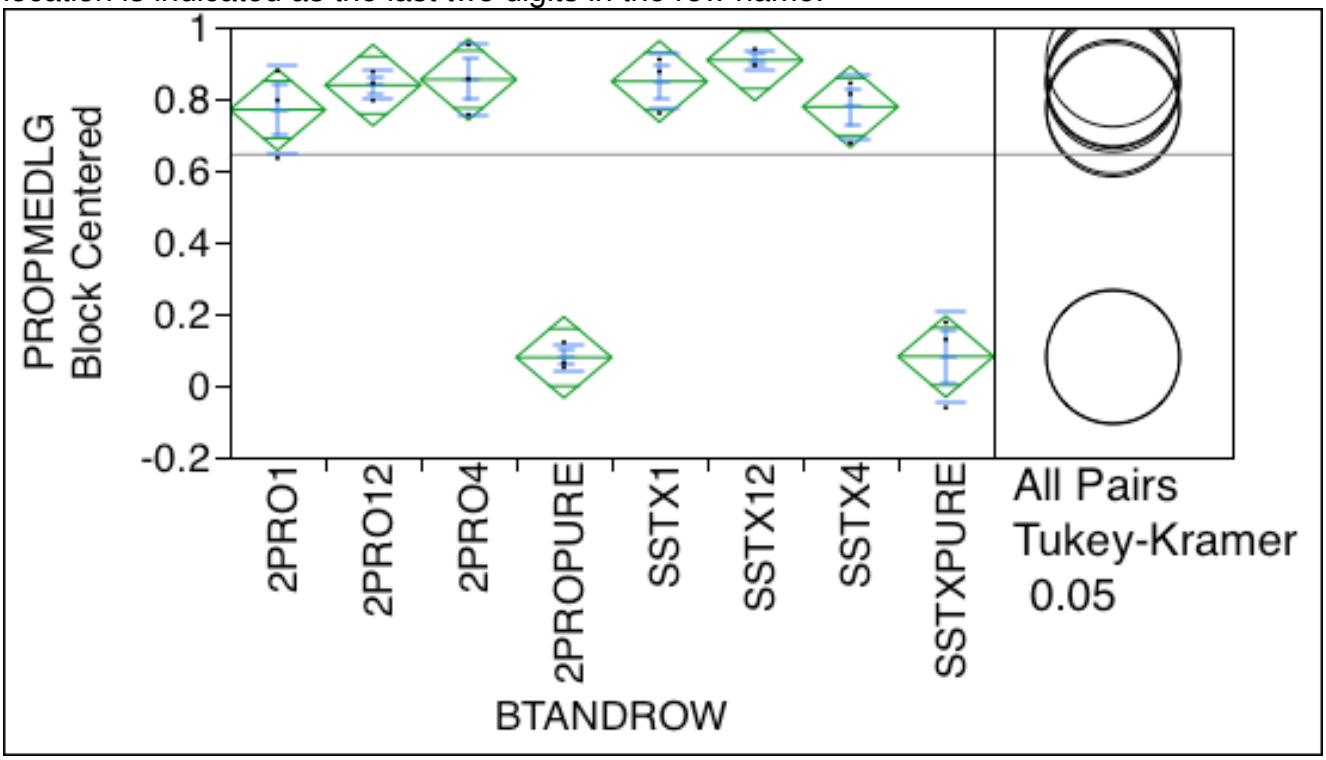
Mean separations (Tukey's HSD) 0.05 level of probability

Proportion of CEW larvae that were small by Bt type and row**.**

Level			**Mean**
2PROPURE	A		0.92129630
SSTXPURE	A		0.91880342
2PRO1		B	0.23059006
SSTX4		B	0.22222222
2PRO12		B	0.16199813
SSTX1		B	0.15138889
2PRO4		B	0.14615105
SSTX12		B	0.09184727

CEW Analysis 4. Mean proportion of medium and large CEW larvae recovered by Bt type and refuge row. The data include sampling in the pure Bt block. Finding: A significantly higher proportion of larvae recovered from the refuge rows were medium and large. THIS IS BASICALLY THE INVERSE OF ANALYSIS 3.

Mean PROPORTION of medium and large CEW by row. This analysis includes rows in the solid Bt block and refuge rows 1, 4 and 12. “2PROPURE” is Double Pro pure Bt block and SSTXPURE is the pure stand of SmartStax 5% refuge in a bag. Refuge row location is indicated as the last two digits in the row name.



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
BTANDROW	7	2.5897371	0.369962	44.3118	<.0001*
REP	2	0.0521030	0.026052	3.1203	0.0757
Error	14	0.1168871	0.008349		
C. Total	23	2.7587273			

Means and Standard Deviations

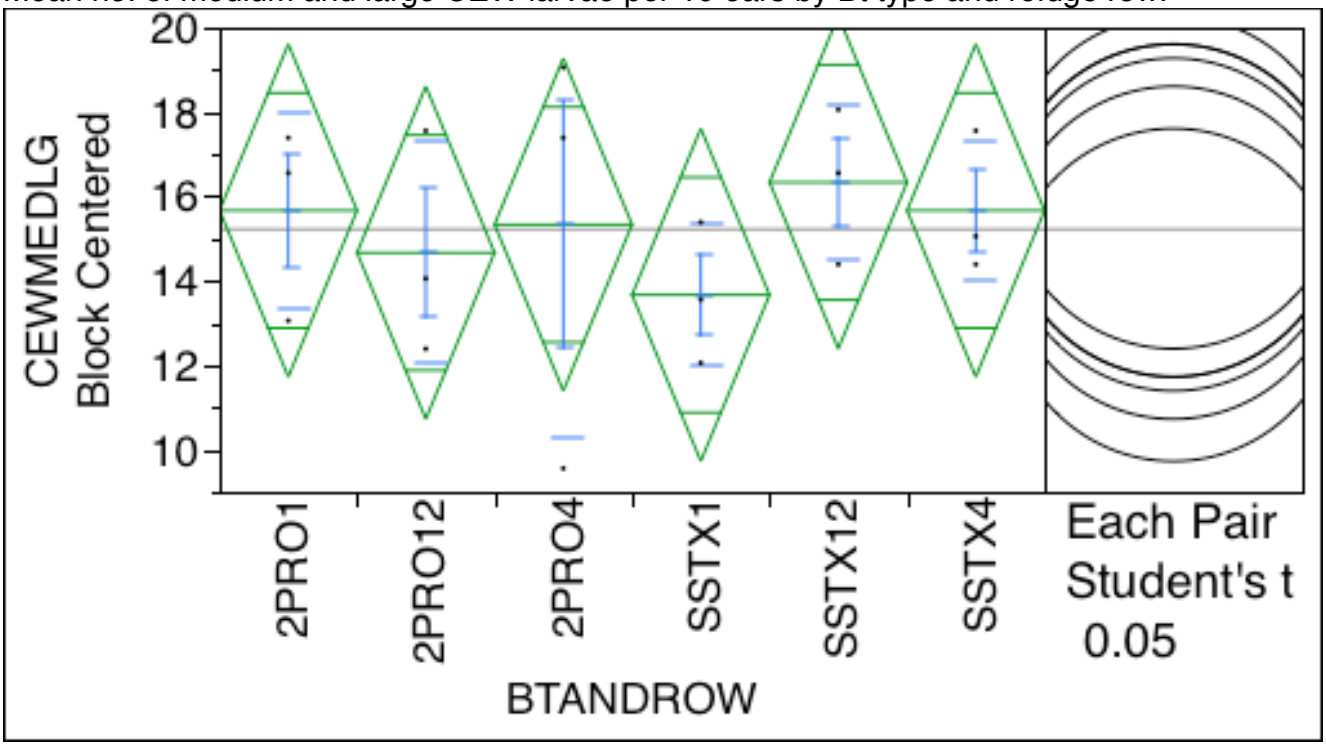
Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
2PRO1	3	0.769410	0.123692	0.07141	0.4621	1.0767
2PRO12	3	0.838002	0.040196	0.02321	0.7381	0.9379
2PRO4	3	0.853849	0.097739	0.05643	0.6111	1.0966
2PROPURE	3	0.078704	0.036902	0.02131	-0.0130	0.1704
SSTX1	3	0.848611	0.078558	0.04536	0.6535	1.0438
SSTX12	3	0.908153	0.026499	0.01530	0.8423	0.9740
SSTX4	3	0.777778	0.089155	0.05147	0.5563	0.9993
SSTXPURE	3	0.081197	0.125664	0.07255	-0.2310	0.3934

Mean separation: Tukey's HSD 0.05 level of probability

Level			**Mean**
SSTX12	A		0.90815273
2PRO4	A		0.85384895
SSTX1	A		0.84861111
2PRO12	A		0.83800187
SSTX4	A		0.77777778
2PRO1	A		0.76940994
SSTXPURE		B	0.08119658
2PROPURE		B	0.07870370

CEW Analysis 5. Mean number of medium and large CEW larvae per ten ears by Bt type and refuge row. This analysis excludes data from within the solid Bt block. No significant differences were found.

Mean no. of medium and large CEW larvae per 10 ears by Bt type and refuge row.



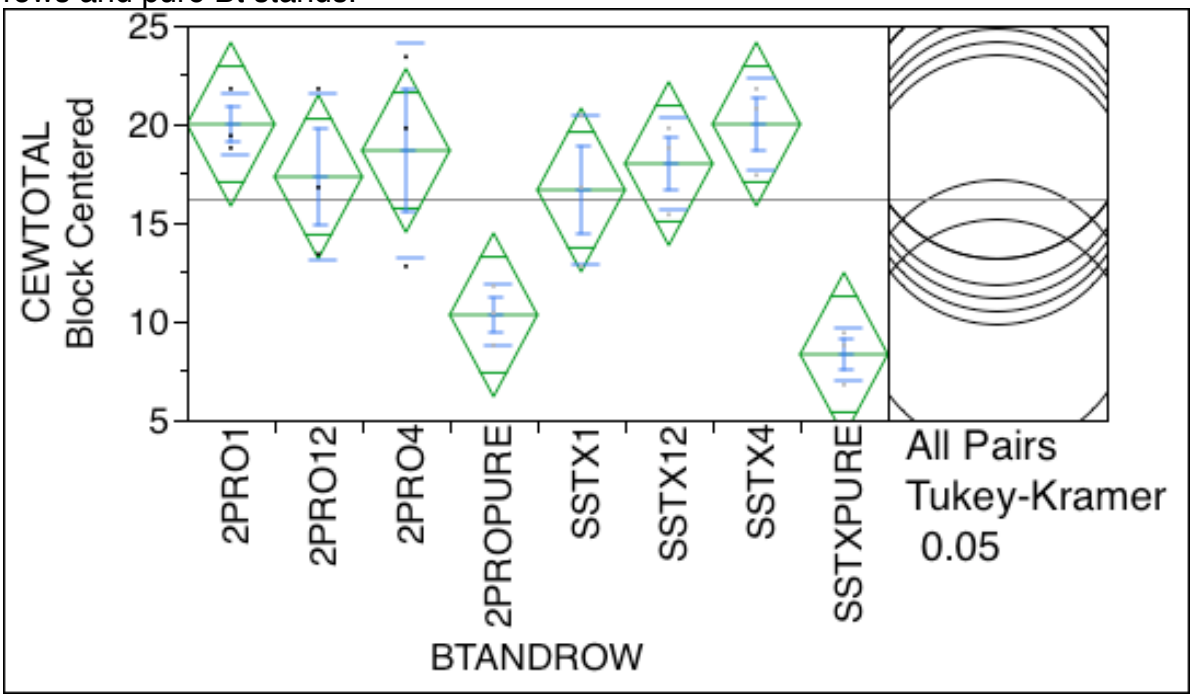
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
BTANDROW	5	13.11111	2.6222	0.2793	0.9141
REP	2	84.11111	42.0556	4.4793	0.0408*
Error	10	93.88889	9.3889		
C. Total	17	191.11111			

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
2PRO1	3	15.6667	2.29936	1.3275	9.955	21.379
2PRO12	3	14.6667	2.63699	1.5225	8.116	21.217
2PRO4	3	15.3333	5.07262	2.9287	2.732	27.934
SSTX1	3	13.6667	1.66944	0.9639	9.520	17.814
SSTX12	3	16.3333	1.84341	1.0643	11.754	20.913
SSTX4	3	15.6667	1.66944	0.9639	11.520	19.814

CEW Analysis 6. Mean total number of CEW larvae recovered per ten ears in refuge rows and pure Bt stands.



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
BTANDROW	7	408.00000	58.2857	5.1892	0.0044*
REP	2	170.08333	85.0417	7.5713	0.0059*
Error	14	157.25000	11.2321		
C. Total	23	735.33333			

Replication Means

REP	Mean	Number
1	19.7500	8
2	13.3750	8
3	15.3750	8

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
2PRO1	3	20.0000	1.58278	0.9138	16.068	23.932
2PRO12	3	17.3333	4.21369	2.4328	6.866	27.801
2PRO4	3	18.6667	5.40110	3.1183	5.250	32.084
2PROPURE	3	10.3333	1.50174	0.8670	6.603	14.064
SSTX1	3	16.6667	3.81404	2.2020	7.192	26.141
SSTX12	3	18.0000	2.29242	1.3235	12.305	23.695
SSTX4	3	20.0000	2.29242	1.3235	14.305	25.695
SSTXPURE	3	8.3333	1.37121	0.7917	4.927	11.740

Mean separations, Tukey's HSD 0.05 level of probability

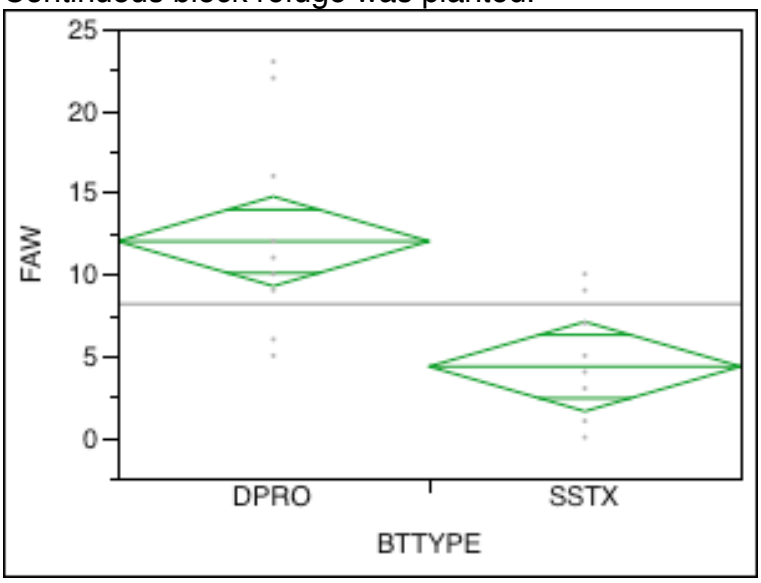
Level				**Mean**
2PRO1	A			20.000000
SSTX4	A			20.000000
2PRO4	A	B		18.666667
SSTX12	A	B		18.000000
2PRO12	A	B	C	17.333333
SSTX1	A	B	C	16.666667
2PROPURE		B	C	10.333333
SSTXPURE			C	8.333333

Because we did not find any ability for either type of Bt corn to protect against corn earworm in strip refuges, we halted further data collection for corn earworm and concentrated our later efforts on fall armyworm.

PART II. Fall Armyworm

FAW Analysis 1 compares the number of FAW larvae recovered on 19 - 20 August (dough stage) from the refuge adjacent to SmartStax and DoublePro solid planting (12 rows of Bt corn). 35 adjacent ears were harvested per refuge row. Data are for combined refuge rows 1, 2, 4, and 12 (hence 140 ears per Bt type x 3 replications = 420 ears per Bt type.) Data from inside the solid Bt block plantings are not included in this analysis. Findings: SSTX had significantly fewer insects in the refuge than did DoublePro. This may result from the presence of Cry1F in SSTX.

FAW larvae per 35 ears in REFUGE ROWS of SmartStax and DoublePro on 8/20/13. Continuous block refuge was planted.



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
BTTYPE	1	352.66667	352.667	16.8423	0.0005*
Error	22	460.66667	20.939		
C. Total	23	813.33333			

Means for Oneway Anova

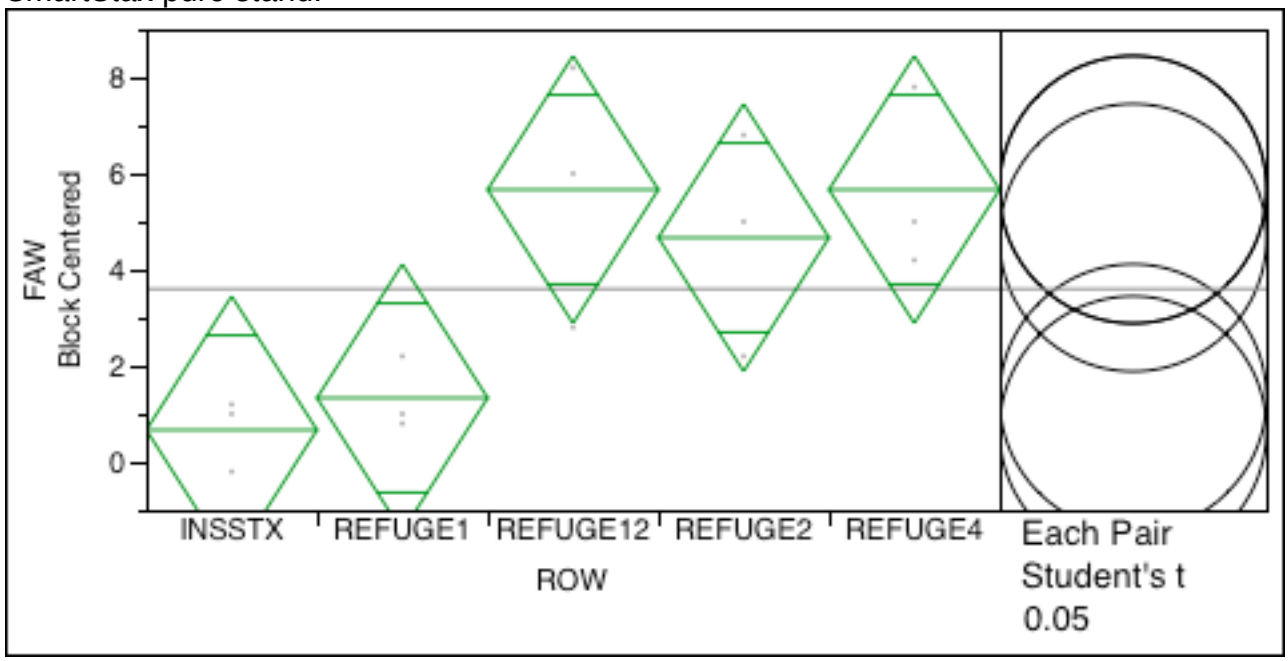
Level	Number	Mean	Std Error	Lower 95%	Upper 95%
DPRO	12	12.0000	1.3210	9.2605	14.740
SSTX	12	4.3333	1.3210	1.5938	7.073

t Test
SSTX-DPRO
Assuming equal variances

Difference	-7.667	t Ratio	-4.10393
Std Err Dif	1.868	DF	22
Upper CL Dif	-3.792	Prob > ltl	0.0005*
Lower CL Dif	-11.541	Prob > t	0.9998
Confidence	0.95	Prob < t	0.0002*

FAW Analysis 2A: Number of FAW larvae recovered on 19 – 20 August (dough stage) in the solid SSTX Bt block planting and adjacent refuge rows 1, 2, 4 and 12. Each mean is composed of 35 ears per row x 3 replications += 105 ears. Finding: this followed an expected trend for fewer larvae in rows closest to the solid Bt planting. It appears that SSTX contributed to lower FAW numbers by wide protection (see analysis 1) and additional specific protection to nearby refuge rows, at least refuge row 1.

SmartStax: FAW Per 35 Ears By ROW. Treatment “INSSTX” is the larval count in the SmartStax pure stand.



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
ROW	4	70.26667	17.5667	4.0229	0.0446*
REP	2	36.40000	18.2000	4.1679	0.0575
Error	8	34.93333	4.3667		
C. Total	14	141.60000			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
INSSTX	3	0.66667	1.2065	-2.115	3.4488
REFUGE1	3	1.33333	1.2065	-1.449	4.1154
REFUGE12	3	5.66667	1.2065	2.885	8.4488
REFUGE2	3	4.66667	1.2065	1.885	7.4488
REFUGE4	3	5.66667	1.2065	2.885	8.4488

Replication Means

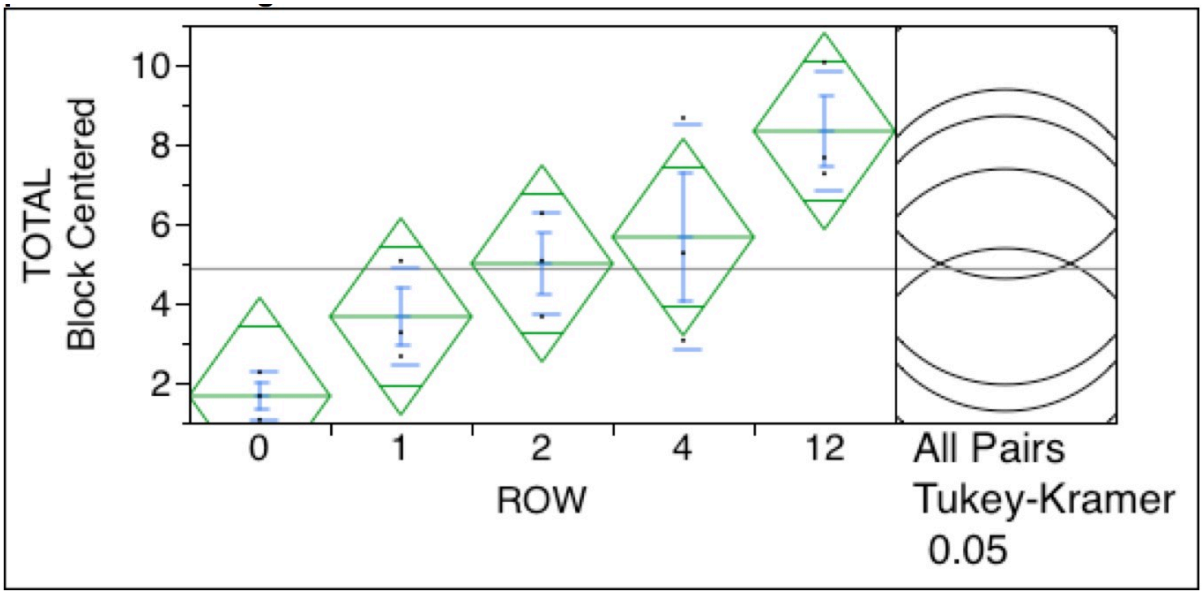
REP	Mean	Number
1	2.60000	5
2	5.80000	5
3	2.40000	5

Mean Separation (t-test)

Level				Mean
REFUGE12	A			5.6666667
REFUGE4	A			5.6666667
REFUGE2	A	B		4.6666667
REFUGE1		B	C	1.3333333
INSSTX			C	0.6666667

Levels not connected by same letter are significantly different.

FAW Analysis 2B: Number of FAW larvae recovered on 6 September (early dent stage) in the solid SSTX Bt block planting and adjacent refuge rows 1, 2, 4 and 12. Each mean is composed of 10 naturally infested ears per row x 3 replications = 30 ears.



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
ROW	4	73.06667	18.2667	5.2692	0.0223*
REP	2	14.93333	7.4667	2.1538	0.1785
Error	8	27.73333	3.4667		
C. Total	14	115.73333			

Block Means

REP	Mean	Number
1	6.20000	5
2	4.60000	5
3	3.80000	5

Means and Std Deviations

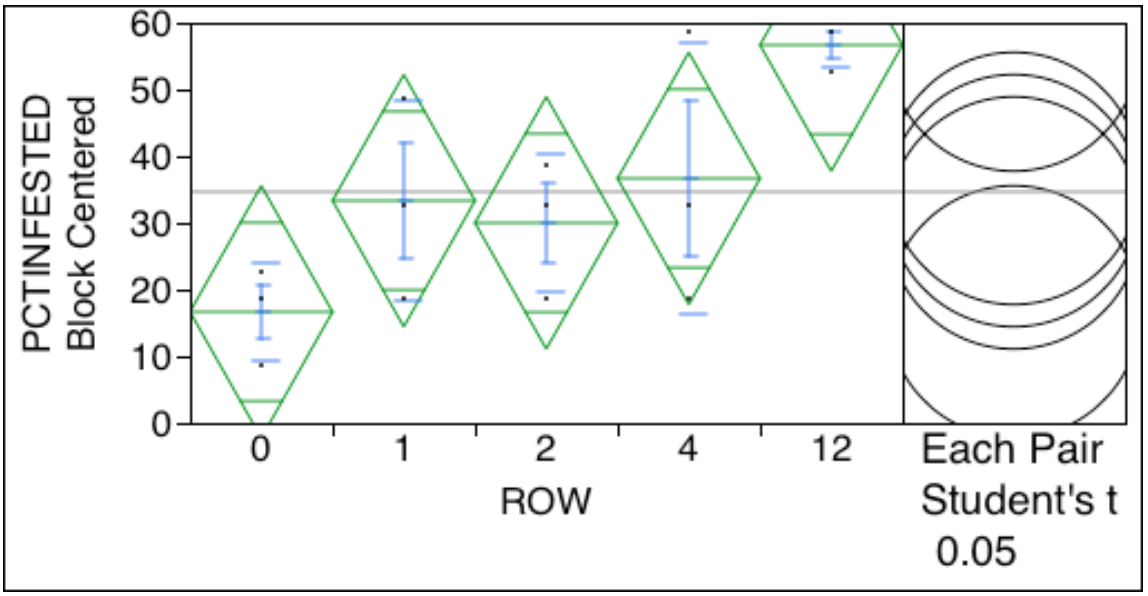
ROW	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
0	3	1.66667	0.60000	0.3464	0.176	3.157
1	3	3.66667	1.24900	0.7211	0.564	6.769
2	3	5.00000	1.30128	0.7513	1.767	8.233
4	3	5.66667	2.82135	1.6289	-1.342	12.675
12	3	8.33333	1.51438	0.8743	4.571	12.095

Mean separation, t-test

Level			Mean
12	A		8.3333333
4	A	B	5.6666667
2	A	B	5.0000000
1	A	B	3.6666667
0		B	1.6666667

Levels not connected by same letter are significantly different.

FAW Analysis 2C: Mean proportion of ears infested with FAW larvae on 6 September (early dent stage) in the solid SSTX Bt block planting and adjacent refuge rows 1, 2, 4 and 12. Each mean is composed of 10 naturally infested ears per row x 3 replications = 30 ears.



Analysis of Variance for arcsine of the square root transformation of proportion of ears infested per row.

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
ROW	4	0.30718482	0.076796	3.0432	0.0844
REP	2	0.13578927	0.067895	2.6904	0.1278
Error	8	0.20188339	0.025235		
C. Total	14	0.64485748			

Block Means

REP	Mean	Number
1	46.0000	5
2	32.0000	5
3	26.0000	5

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
0	3	16.6667	7.2111	4.163	-1.25	34.580
1	3	33.3333	15.0111	8.667	-3.96	70.623
2	3	30.0000	10.2632	5.925	4.50	55.495
4	3	36.6667	20.2978	11.719	-13.76	87.089
12	3	56.6667	3.4641	2.000	48.06	65.272

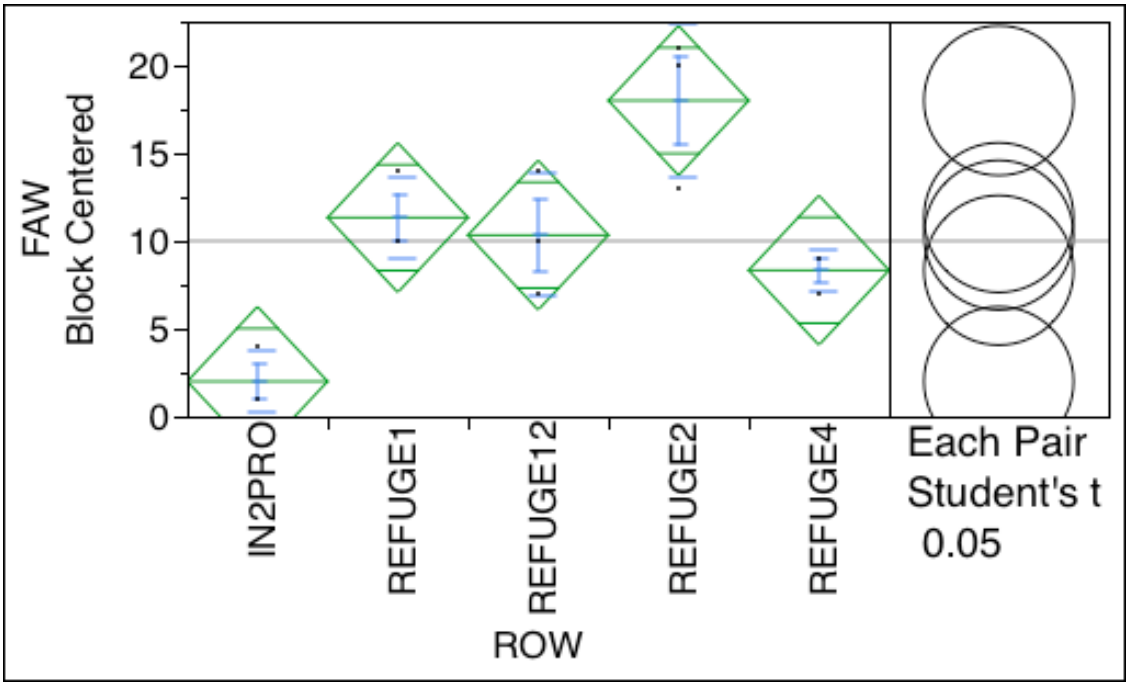
Row mean separations, t-test. Separations shown are for proportion of infested ears as transformed by the arcsine of the square root transformation.

Row			Mean
12	A		56.666667
4	A	B	36.666667
1	A	B	33.333333
2	A	B	30.000000
0		B	16.666667

Levels not connected by same letter are significantly different.

FAW Analysis 3: Number of FAW larvae recovered on 19 - 20 August (dough stage) in the solid DOUBLEPRO Bt block planting and adjacent refuge rows 1, 2, 4 and 12. Each mean is composed of 35 ears per row x 3 replications = 105 ears. Finding: Double Pro solid Bt blocks provide no protection for any of the adjacent rows. The high number of insects in refuge row 2 reflects a hotspot in one replication where 23 FAW larvae were found in 35 ears.

DOUBLE PRO FAW Per 35 Ears by Row. IN2PRO indicates “in the pure block of Double Pro”.



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
ROW	4	398.00000	99.5000	9.7073	0.0037*
REP	2	120.00000	60.0000	5.8537	0.0272*
Error	8	82.00000	10.2500		
C. Total	14	600.00000			

Block Means

REP	Mean	Number
1	12.0000	5
2	6.0000	5
3	12.0000	5

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean	Lower 95%	Upper 95%
IN2PRO	3	2.0000	1.73205	1.0000	-2.303	6.303
REFUGE1	3	11.3333	2.30940	1.3333	5.596	17.070
REFUGE12	3	10.3333	3.51188	2.0276	1.609	19.057
REFUGE2	3	18.0000	4.35890	2.5166	7.172	28.828
REFUGE4	3	8.3333	1.15470	0.6667	5.465	11.202

Mean Separation: t-test

Level				Mean
REFUGE2	A			18.000000
REFUGE1		B		11.333333
REFUGE12		B		10.333333
REFUGE4		B		8.333333
IN2PRO			C	2.000000

Levels not connected by same letter are significantly different.

PART III. Assay of qualitative toxin expression in kernels

Pollen Assay 1: Toxins expressing in bulk ground kernels from the top 1/3 of an ear

Five ears per plot from both SmartStax and Double Pro plots were removed from the field at early dent stage and stored for two weeks in a warehouse. Kernels were removed from the top 1/3 of an ear and ground in bulk in a hand blender. The ground fines for each individual ear were subjected to quick strip assays. The strips could detect Cry34, Cry1F, Cry3Bb1 and Cry2Ab2. This was done individually for 5 ears per plot. There were 3 replications.

The test strips, while not quantitative, showed either strong lines with strong coloration or weak lines that were lightly colored. We recorded the type of line for each positive reading and divided these into “strong expression” or “weak expression” categories.

Pollen Table 1. Toxin expression detected in bulk ground kernels in a SmartStax RIB block and refuge rows 1, 2, 4 and 12. Percentage values are of those ears that expressed all of the SmartStax toxins. Five ears per plot x 3 replications. These data are for complete SSTX toxin suite expression only. Incomplete suite expression is presented in Table 2.

Location	N	No. expressing SSTX (Pct. expressing)	Strong SSTX expression	Weak SSTX expression	Pct. strong expression	Pct. weak expression
In SSTX	15	15 (100)	15	0	100	0
Row 1	15	12 (80)	9	3	75	25
Row 2	15	14 (93)	11	3	79	21
Row 4	15	10 (67)	4	6	40	60
Row 12	15	8	0	8	0	100

Pollen Table 2. Lepidoptera toxins detected in ears that did not express all toxins in SmartStax in a SmartStax RIB block and refuge rows 1, 2, 4 and 12.

Location	N	No. not expressing SSTX	No. expressing Cry1F	No. expressing Cry2Ab2	Pct. expressing Cry2Ab2
In SSTX	15	0	0	0	0
Row 1	15	3	0	2	13.3
Row 2	15	1	0	0	0
Row 4	15	5	0	1	6.7
Row 12	15	7	0	1	6.7

Pollen Table 3. Cry2Ab2 toxin expression detected in bulk ground kernels in a Double Pro block and refuge rows 1, 2, 4 and 12. Five ears per plot x 3 replications.

				---------- Cry2Ab2 expression --------			
Location	N	No. expressing Cry2Ab2	Pct. expressing Cry2Ab2	No. Strong	No. Weak	Pct. strong	Pct. weak
In 2Pro	15	15	100	15	0	100	0
Row 1	15	15	100	7	8	46.7	53.5
Row 2	15	10	66.7	5	5	50	50
Row 4	15	7	46.7	2	5	28.6	71.4
Row 12	15	10	66.7	6	4	60	40

Pollen Assay 2: Toxins expressing in individual kernels near the ear tip.

We removed 10 individual kernels from as close as possible to the ear tip of each of 4 ears in refuge row 2 of the 3 SmartStax replications. (This assay was not done for the Double Pro blocks.) There was often some tip damage and associated fungi so we chose the intact kernels closest to the ear tip. Each kernel was assayed with quick strips that detect Cry34, Cry1F, Cry3Bb1 and Cry2Ab2.

Pollen Table 4A. Individual toxin expression in tip kernels in SmartStax refuge row 2. Data include kernels that were positive for all SSTX toxins and those that expressed only a subset of toxins. ***See table 5 for explanation of pyramids vs. single toxins detected.***

Rep	No. ears	No. kernels	Lepidoptera toxins No. of kernels Negative for toxins	Positive for toxins	Percent of kernels positive for Lepidoptera toxins	Total positive detections Cry1F	Cry2Ab2
1	4	40	28	12	30.0	7	9
2	4	40	31	9	22.5	3	6
3	4	40	23	17	42.5	9	9
Refuge row 2 total values / 120 kernels			82	38	31.6	19	24

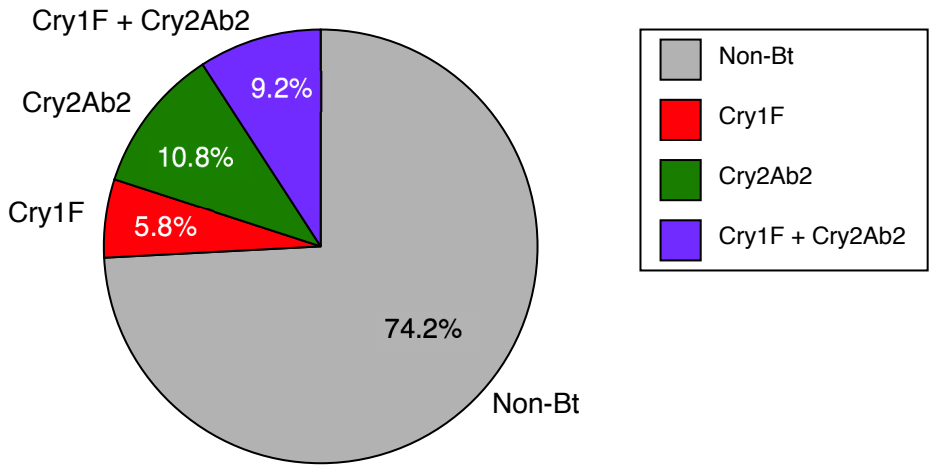
Pollen Table 4B. These are the same data as in Table 4 but with the rootworm toxins presented as well. See table 5 for explanation of pyramids vs. single toxins detected.

			No. of kernels		-- No. of kernels positive for a toxin --			
Rep	No. ears	No. kernels	Negative for toxins	Positive for toxins	Cry34	Cry1F	Cry3Bb1	Cry2Ab2
1	4	40	17	23	5	7	5	9
2	4	40	32	8	3	3	4	6
3	4	40	24	16	5	9	9	9

Pollen Table 5. Expression of single Lepidoptera toxins and a Lepidoptera pyramids in refuge row 2 kernels that contained at least one Lepidoptera toxin. **This table addresses toxins that are apparently segregating as well as those that are not.**

Rep	No. of kernels tested	No. with only Cry1F	No. with only Cry2Ab2 + Cry1A.105	No. with both Cry1F and Cry2Ab2
1	40	3	5	4
2	40	1	4	2
3	40	3	4	5
Pct. of all 120 kernels		5.8	10.8	9.2

Percentage of tip kernels in Refuge Row 2 with various Bt toxin profiles



10 tip kernels / ear x 4 ears per plot x 3 replications = 120 kernels