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ARTHROPOD MANAGEMENT AND APPLIED ECOLOGY

Impact of Various Bt Cotton Traits and the Application of an Insecticide on the Within Plant Distribution of *Helicoverpa zea* (Lepidoptera: Noctuidae) Larvae and Injured Floral Structures

D.D. Kerns*, D.L. Kerns, G.M. Lorenz, A.L. Catchot, and S.D. Stewart

ABSTRACT

Previous studies have indicated that the expression of insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt) in cotton can have a significant influence on the behavior of bollworm larvae (*Helicoverpa zea*, Lepidoptera: Noctuidae). This suggests that the particular Bt protein produced by a cotton variety may need to be considered when determining the most ideal scouting methods to utilize for bollworm. Non-Bt, WideStrike (producing Cry1Ac + Cry1F Bt insecticidal proteins), and Bollgard II (Cry1Ac + Cry2Ab) cotton varieties were planted and either treated with an insecticide or left untreated. The presence of *H. zea* larvae and their feeding injury were recorded according to their location in the canopy and type of floral structure where they were found. Results from comparison of larval and injury distributions indicated no significant differences between the different cotton varieties tested, and that insecticide treatment had minimal impact on this distribution. Larval size was generally associated with location in the canopy, suggesting that larvae tend to move towards the middle of the canopy as they age. The effect of different Bt cotton technologies appears to associate with how quickly larvae move to preferred feeding sites rather than their preference for particular feeding sites. These results suggest that scouting methods could be standardized independently of the presence of a Bt cotton trait or previous insecticide application. Focusing scouting efforts on the middle portion of the canopy (i.e., nodes 6-9) should increase the detection of small larvae and 'fresh' injury and be less influenced by previous insecticide applications.

Helicoverpa zea (Lepidoptera: Noctuidae), commonly referred to as bollworm or corn earworm, has historically been considered a major pest of cotton in the United States (Luttrell 1994). Female moths have a preference for oviposition on host plants while they are flowering, thus *H. zea* is commonly a late season pest of cotton (Johnson et al. 1975). Typically, small larvae feed on small squares in the upper canopy before they increase in size and begin to feed in lower regions of the canopy on larger floral structures such as bolls (Wilson et al. 1980, Reese et al. 1981, Farrar and Bradley 1985).

Transgenic cotton producing one or more insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt) was commercialized for the control of important lepidopteran pests (Fleming et al. 2018, Kerns et al. 2018). The prominence of *H. zea* as a pest in cotton increased following the widespread use of Bt cotton as a standard insect management practice, in part because it is inherently less susceptible to the Bt proteins expressed in Bt cotton compared with the tobacco budworm, *Chloridea virescens* (Lepidoptera: Noctuidae) (Luttrell and Jackson 2012). Thus, the application of a supplemental insecticide to Bt cotton is sometimes necessary to maintain adequate management of bollworm despite the substantial benefit the technology provides as a management tool (Reisig et al. 2019).

The scouting of Bt cotton for the presence of bollworm remains an important component in management of this pest. Previous studies suggest that the expression of Bt proteins in cotton plant tissues can significantly impact the behavior and

D.D. Kerns* and S.D. Stewart, Dept. of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN; D.L. Kerns, Dept. of Entomology, Texas A&M University, College Station, TX; G.M. Lorenz, Dept. of Entomology and Plant Pathology, University of Arkansas, Fayetteville, AR; and A.L. Catchot, Dept. of Biochemistry, Molecular Biology, Entomology, and Plant Pathology, Mississippi State University, Starkville, MS.
*Corresponding author: dkerns1@vols.utk.edu

plant canopy distribution of *H. zea* larvae (Bommireddy et al. 2007, Gore et al. 2002, Gore et al. 2003){Gore, 2002 #232;Gore, 2003 #236;Jackson, 2010 #237;Bommireddy, 2007 #257}. In contrast, Jackson et al. (2010) found no difference in vertical larval movement on non-Bt and transgenic Bt cotton expressing Cry1F + Cry1Ac. Several factors have been identified as variables that can influence the behavior of *H. zea* larvae in cotton. Larvae of *H. zea* exhibit preference for diets containing lower concentrations of Bt proteins (Gore et al. 2005) and the concentration of Bt proteins in Bt cotton varies both spatially and temporally (Kranthi et al. 2005, Siebert et al. 2009, Sivasupramaniam et al. 2014). This may explain why larvae in Bt cotton move to lower canopy regions faster than in non-Bt cotton, possibly in response to the concentration gradient of Bt proteins throughout the plant (Gore et al. 2002). The window of time during the growing season at which an infestation occurs has also been identified as a possible contributing factor to the varying behavior and canopy distribution of *H. zea* larvae. Vertical distribution of bollworm eggs was observed to favor the upper portion of the canopy later in the season, thus impacting the subsequent distribution of newly eclosed larvae (Braswell et al. 2019).

Since Bt cotton was first commercialized, and as new Bt technologies have been introduced, some pest advisors have deviated from standardized scouting methods for bollworm in favor of methods they feel are more suitable for Bt cotton. This may involve focusing on lower regions in the canopy, on small bolls, or the bloom tags (floral remnants) of bolls rather than more traditional systematic and top-down scouting. Consequently, currently recommended treatment thresholds may not be suitable when making treatment decisions based on modified sampling procedures. In addition, the various Bt cotton products differ in their ability to control bollworm (Kerns et al. 2018), and thus, egg or larval thresholds should and often do differ among them (e.g., Stewart and McClure 2020). These factors can create uncertainty and confusion when making insecticide treatment decisions for bollworm in systems where multiple Bt cotton technologies are deployed. This uncertainty is further compounded where *H. zea* is developing resistance to some Bt insecticidal proteins present in cotton (Yang et al. 2017, Yang et al. 2019). Ideally, there would be a standardized method of scouting and making insecticide treatment decisions that would be suitable for Bt and non-Bt cotton varieties. Towards advancing development of this standardized method, the goal of this project was to determine how Bt technologies may affect the distribution of *H. zea* larvae and their injury within the canopy of cotton.

MATERIALS AND METHODS

Experimental Design . In 2018, eight row main plots of non-Bt Phytogen 425 RF (Corteva Agriscience, Indianapolis, IN), Phytogen 444 WRF (WideStrike, Cry1F + Cry1Ac, Corteva Agriscience, Indianapolis, IN), and Deltapine 1646 B2XF (Bollgard II, Cry1Ac + Cry2Ab, Bayer CropScience, St. Louis, MO) cotton varieties were planted within a randomized complete block design with four replications on 12 June in Jackson, TN. It was expected that these varieties would provide variable bollworm infestation levels owing to the presence (WideStrike, Bollgard II) or lack of Bt traits, and that the different Bt traits may also affect the behavior of *H. zea* larvae. Row spacing was 97 cm, plots were 12 m long, and 13.3 seeds were planted per meter in each row. Main plots were divided into four row sub-plots that were either treated or not with a foliar application of chlorantraniliprole (60 g ai/ha, Prevathon, FMC Corporation, Philadelphia, PA). This application was made on 21 August, once primarily small *H. zea* larvae were detected at a density of greater than four larvae per 100 plants.

The exact same experimental design, varieties, row spacing, and planting rate were used in 2019, but the experiment was repeated at multiple locations. Cotton was planted on 30 April, 16 May, and 4 June in College Station (TX), Tillar (AR), and Jackson (TN), respectively. Plot length varied from 12-14 m. In 2019, lambda-cyhalothrin (35.7 g ai/ha, Warrior II, Syngenta Corporation, Wilmington, DE) was used rather than chlorantraniliprole to allow for greater post-treatment survival of bollworm. The insecticide applications were made on 17 July in Texas, 24 July in Arkansas, and 15 August in Tennessee using the same criteria used in 2018 to initiate treatment. Plots were managed with selective insecticides for other pests as needed to prevent fruit loss.

Sampling Procedures. In 2018, sampling was performed on 26 August. In 2019, samples were taken on 22 July, 30 July, and 20 August in Texas, Arkansas, and Tennessee, respectively. In both years, sampling for *H. zea* larvae and injury was done when cotton was near physiological cutout (i.e., four to five nodes above white flower [NAWF]), and thus plants had a near maximum number of total nodes and ample numbers of squares, blooms, and bolls of various sizes. Varieties were chosen because they had similar growth patterns and maturity. Obvious differences in fruiting patterns and retention were not evident; therefore we did not collect data on fruit retention prior to initiating the experiment. A low level of square and boll injury was apparent prior to making the insecticide application, particularly in 2019 and in the non-Bt variety resulting from sub-threshold infestations of *H. zea*.

After a preliminary assessment, subplots treated with chlorantraniliprole in 2018 were not sampled because this application very effectively reduced the number of *H. zea* larvae and injury levels to negligible levels. All subplots were sampled in 2019. The center two rows of subplots were sampled by selecting five consecutive plants from three randomly chosen spots. These plants were cut at the base of the plant and removed to the edge of the field. However, spindly or grossly atypical plants were avoided because they would make mapping the location of larvae and injury difficult. Portable tables and tents were placed at the field edge, and the presence of *H. zea* larvae and injury for each of the 15 plants from a subplot were mapped immediately following removal from the field.

Mapping consisted of recording the node where a larva or injured floral structure was found. Larvae were categorized as either small (1^{st} and 2^{nd} instar, 1-5 mm), medium (3^{rd} and 4^{th} instar, 6-16 mm), or large (5^{th} instar or larger, >16 mm). A floral structure was considered injured if the square or boll 'wall' had been penetrated. Injury to flowers also included signs of feeding consistent with *H. zea*. We categorized whether the larva or injury was found on a square, candle square, white flower, pink flower, bloom tag boll, small boll, or boll. A candle square is the last stage of development of a square before it opens as a flower, thus all squares in the candle stage were categorized as "candle squares" and all other squares in prior stages of development were categorized together as "squares". A cotton flower only persists for one day as a "white flower", after which the white petals turn pink and begins to wither. "Pink flowers" were those that retained some moisture and pink coloration, typically for two to four days after flowering. After pink flower, the dried bloom remnants either fall off the boll or remain stuck to the tip of the boll (i.e., bloom tag). Bolls that retained a bloom tag were categorized as "bloom tag bolls" and bolls that were similar in size but had no bloom tag were categorized as small bolls. Any larger bolls were categorized as "bolls".

Analyses. The number of larvae (by larval size) and the total amount of injury (by floral structure) were calculated for each subplot. For analyses, larval location and injury were categorized by canopy level (top, middle, bottom). The top five nodes of plants were designated as the top canopy, nodes six through nine were designated as the middle canopy, and nodes below the ninth node were considered the bottom canopy. After preliminary analyses, it was decided to more coarsely categorize larval location and injury for floral structures as square (square and candle square), flower (white flower and pink flower), or boll (bloom tag boll, small boll, boll), rather than by the finer categorizations chosen when the data were collected. This was done because some of the sample sizes for the finer categorizations were too small to make any meaningful comparisons. Similarly, initial analyses indicated no significant differences in the distribution of larvae or injury between Bollgard II and WideStrike cotton. Thus, Bollgard II and WideStrike cotton plots were labeled as a single, indistinguishable "Bt" treatment for all analyses to increase statistical power.

Data was analyzed using GLIMMIX procedures (α=0.05, SAS ver. 9.4, SAS Institute, Cary, NC). Fixed effects included in the statistical models included Bt trait (non-Bt and Bt), insecticide treatment (treated or not), canopy level (top, middle, bottom), floral structure (square, flower, boll), larva size (small, medium, large) and all their interactions. Depending on the comparisons being made, models did not include all fixed effects, and variations of these fixed effects are specified in Table 1. Random effects in the models included location, appropriate interactions between locations and other effects, and replication as a nested effect within other model effects (Table 1). When fixed effects were determined to be significant, a Tukey HSD test was used for mean separation.

Table 1. List of all main effects, interactions between main effects, and random effects that were included in each statistical model that was part of the analysis for this study

Fixed and Random Effects for All Models			
Models	**Main Effects**	**Main Effect Interactions**	**Random Effects**
1[yw]	Trait Insecticide Canopy	All 2- and 3-way	Location, Location*Trait, Location*Insecticide, Location*Trait*Insecticide, Location*Trait*Insecticide*Canopy, Rep(Location*Trait*Insecticide)
2[yw]	Trait Insecticide Structure	All 2- and 3-way	Location, Location*Trait, Location*Insecticide, Location*Trait*Insecticide, Location*Trait*Insecticide*Structure, Rep(Location*Trait*Insecticide)
3[zw]	Trait Insecticide Canopy	All 2-and 3-way	Location, Location*Trait, Location*Insecticide, Location*Trait*Insecticide, Location*Trait*Insecticide*Canopy, Rep(Location*Trait*Insecticide)
4[zw]	Trait Insecticide Structure	All 2-way	Location, Location*Trait, Location*Insecticide, Location*Trait*Insecticide, Location*Trait*Insecticide*Structure, Rep(Location*Trait*Insecticide)
5[yx]	Trait Canopy	Trait*Canopy	Location, Location*Trait, Location*Trait*Canopy, Rep(Location*Trait)
6[yx]	Trait Structure	Trait*Structure	Location, Location*Trait, Location*Trait*Canopy, Rep(Location*Trait)
7[zx]	Trait Canopy	Trait*Canopy	Location, Location*Trait, Location*Trait*Canopy, Rep(Location*Trait)
8[zx]	Trait Structure	Trait*Structure	Location, Location*Trait, Rep(Location*Trait)
9, 10, 11[zw]	Trait Larval Size	Trait*Size	Location, Location*Trait, Rep(Location*Trait)

[z,y] **Larval counts and injured structure counts, respectively.**

[x,w] **2019 alone or 2018 and 2019 combined, respectively.**

For data collected in 2019, the distribution of injured structures throughout the canopy or between different floral structure types was analyzed using two separate models (Table 1; Models 1 and 2). The distribution of larvae based on canopy level and floral structure type in 2019 was also analyzed as two separate models (Table 1; Models 3 and 4). Fixed effects were the same as the first two models that were previously discussed, however, no three-way interaction was included in Model 4 due to failure of the model to converge.

Data collected in 2018 and 2019 were analyzed together to evaluate the distribution of injured structures within the canopy and between types of floral structures (Table 1; Models 5 and 6). Insecticide treatment was excluded as a main effect from all models that analyzed data from 2018 because no data on insecticide effects was collected that year due to low survival of larvae in treated plots. A model to analyze the number of observed larvae distributed between different canopy levels was also constructed from compiled 2018 and 2019 data (Table 1; Model 7). Another model was constructed to analyze the distribution of larvae between different floral structures, however, only trait and floral structure type were included in the model as main effects so that the model would converge (Table 1; Model 8). The data from 2018 and 2019 was partitioned by canopy level (top, middle, bottom) and included in three separate models to evaluate larval size distribution in each portion of the canopy (Table 1; Models 9, 10, and 11).

RESULTS

In 2018, no *H. zea* larvae and very little injury was found in preliminary samples of non-Bt cotton that were treated with chlorantraniliprole. Therefore, subplots treated with this insecticide were not sampled. Consequently, data was unbalanced across years, and the results for the effect of insecticide treatment are presented only for 2019. Despite there only being one location in 2018, it was the most heavily infested test, although overall bollworm infestation levels was moderate and lower than normally observed. The mean number (SEM) of injured floral structures observed on 15 plants in non-Bt plots that were not treated with an insecticide was 38.25 ± 12.44, 14.0 ± 3.72, 14.0 ± 2.68, and 24.25 ± 1.49 for Tennessee (2018), Tennessee (2019), Arkansas, and Texas respectively.

Vertical Distribution of Injury and Larvae in the Canopy. As expected, the non-Bt cotton variety had considerably more injured floral structures than the Bt varieties, regardless of whether data were combined across years or not (Table 2). The application of a pyrethroid insecticide in 2019 did not significantly reduce the total amount of injury caused by *H. zea* larvae (Table 2). Less injury was observed in the bottom portion of the canopy compared with the middle and upper portions, and again, this pattern was similar whether data were combined across years or not (Table 2). The effects of canopy level and insecticide were found to have a significant interaction (Table 2). Injury in the upper canopy was significantly reduced by approximately 54% in plots that received a pyrethroid treatment (Figure 1). In contrast, there was a slight, but not statistically significant, increase in the mean number of injured structures in the bottom portion of the canopy when a pyrethroid insecticide was applied. No other two-way or three-way interactions were observed.

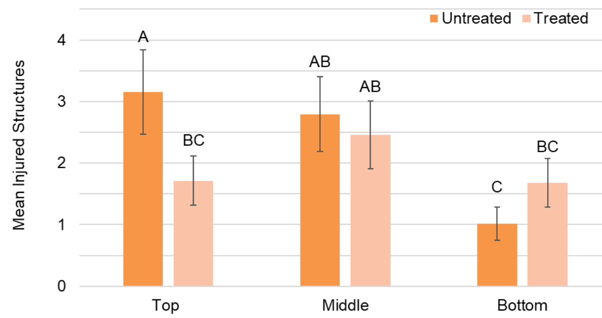

Figure 1. Effect of foliar application of lambda-cyhalothrin on the mean number of injured floral structures in each portion of the canopy across all three cotton traits at all three locations in 2019 (F=3.63; df=2,8; p=0.0380). Bars labeled with the same letter are not significantly different (p>0.05, Tukey HSD).

Table 2. Effect of Bt trait, foliar insecticide treatment, or canopy level on the mean number of injured floral structures in either 2018 and 2019 or 2019 alone

Mean Number of Injured Foliar Structures				
Main Effect	**Treatments**	**2018 and 2019**	**2019**	
Trait	**Non-Bt**	**17.73a**	**13.35a**	
	Bt	**4.14b**	**2.7b**	
Insecticide	**Treated**	**---**	**5.76a**	
	Untreated	**---**	**6.24a**	
Canopy	**Top**	**3.62a**	**2.32a**	
	Middle	**3.64a**	**2.62a**	
	Bottom	**1.77b**	**1.3b**	
Type III Tests of Fixed Effects				
Year	**Main Effect**	**F-Value**	**df**	**P-Value**
2018 and 2019[z]	**Trait**	**17.18**	**1, 3**	**0.0255**
	Canopy	**5.96**	**2, 12**	**0.0159**
	Trait*Canopy	**0.46**	**2, 12**	**0.6435**
2019[y]	**Trait**	**26.39**	**1, 2**	**0.0359**
	Insecticide	**0.24**	**1, 2**	**0.6744**
	Canopy	**7.01**	**2, 16**	**0.0065**
	Trait*Insecticide	**0.27**	**1, 2**	**0.6523**
	Trait*Canopy	**0.29**	**2, 16**	**0.7531**
	Insecticide*Canopy	**4.04**	**2, 16**	**0.0380**
	Trait*Insecticide*Canopy	**1.26**	**2, 16**	**0.3099**

[z,y] Statistical Models 5 and 1 respectively (Table 1).

Means within main effect and year(s) columns followed by the same letter are not significantly different (p >0.05, Tukey HSD).

Larval numbers were low compared with the numbers of injured floral structures, but similar to injury, the vast majority of larvae were found in the non-Bt cotton. This was true regardless of whether the data were analyzed across years or not (Table 3). There was no significant difference between the number of larvae observed in cotton treated with a pyrethroid and cotton that was not treated (Table 3). Canopy level had a significant effect on the number of observed larvae (Table 3), with most larvae found in the top and, in particular, the middle portion of the canopy. Interactions were not observed (Table 3).

The Bt trait did not have a significant influence on the number of larvae observed in each individual portion of the canopy (Table 4). However, the trend in each part of the canopy matched the overall observation (Table 3) of fewer larvae in Bt cotton than in non-Bt cotton. Mostly small and medium=sized larvae were found, regardless of canopy level, with more medium-sized larvae observed in the middle canopy than small and large larvae (Table 4). No interaction between trait and larval size was observed in any portion of the canopy (Table 4).

Distribution of Injury and Larvae Among Floral Structures. As seen with the previous analyses, more injured floral structures were observed in non-Bt cotton compared to cotton with Bt traits, and no significant difference in the total number of injured structures was observed between plots that were treated with a pyrethroid insecticide and plots that were not (Table 5). Squares and bolls were the most commonly observed injured structures (Table 5). No significant interactions among the main effects were found (Table 5).

Also as seen with the previous analyses, larvae were more common in the non-Bt compared with Bt cotton, and there was no significant difference in the number of larvae found in plots that were treated with a pyrethroid insecticide compared with those not treated (Table 6). Significantly more larvae were found on bolls than squares or flowers when analysis were conducted across both years or for 2019 alone (Table 6). The mean number of larvae found on squares did not statistically separate from the mean number of larvae found on flowers. Two-way and three-way interactions of main effects on larval numbers were not significant (Table 6).

Table 3. Effect of Bt trait, foliar insecticide treatment, or canopy level on the mean number of *H. zea* larvae in either 2018 and 2019 or 2019 alone

		Mean Number of Larvae		
Main Effect	**Treatments**	**2018 and 2019**	**2019**	
Trait	**Non-Bt**	**3.48a**	**2.31a**	
	Bt	**0.93b**	**0.36b**	
Insecticide	**Treated**	**---**	**0.84a**	
	Untreated	**---**	**0.99a**	
Canopy	**Top**	**0.56ab**	**0.29ab**	
	Middle	**0.99a**	**0.54a**	
	Bottom	**0.38b**	**0.18b**	
	Type III Tests of Fixed Effects			
Year	**Main Effect**	**F-Value**	**df**	**P-Value**
2018 and 2019[z]	**Trait**	**19.51**	**1, 3**	**0.0215**
	Canopy	**5.28**	**2, 12**	**0.0227**
	Trait*Canopy	**0.96**	**2, 12**	**0.4088**
2019[y]	**Trait**	**25.77**	**1, 2**	**0.0367**
	Insecticide	**0.24**	**1, 2**	**0.6734**
	Canopy	**5.93**	**2, 16**	**0.0119**
	Trait*Insecticide	**0.45**	**1, 2**	**0.5723**
	Trait*Canopy	**1.86**	**2, 16**	**0.1880**
	Insecticide*Canopy	**0.77**	**2, 16**	**0.4773**
	Trait*Insecticide*Canopy	**0.17**	**2, 16**	**0.8417**

[z,y] Statistical models 7 and 3 respectively (Table 1).

Means within main effect and year(s) columns followed by the same letter are not significantly different (*p*>0.05, Tukey HSD).

Table 4. Effect of Bt trait and larva size on the mean number of larvae in the top, middle, or bottom portions of the canopy across 2018 and 2019

Mean Number of Larvae				
Main Effect	**Treatments**	**Top**	**Middle**	**Bottom**
Trait	Non-Bt	1.05a	1.59a	0.54a
	Bt	0.15a	0.63a	0.15a
Size	Small	0.33a	0.33b	0.11ab
	Medium	0.16ab	0.59a	0.17a
	Large	0.05b	0.18b	0.04b
Type III Tests of Fixed Effects				
Canopy	**Main Effect**	**F-Value**	**df**	**P-Value**
Top[z]	Trait	9.25	1, 3	0.0558
	Size	5.97	2, 108	0.0035
	Trait*Size	0.88	2, 108	0.4164
Middle[y]	Trait	9.66	1, 3	0.0530
	Size	6.35	2, 108	0.0025
	Trait*Size	0.95	2, 108	0.3897
Bottom[x]	Trait	1.91	1, 3	0.1387
	Size	2.24	2, 108	0.1103
	Trait*Size	0.82	2, 108	0.4432

[z,y,x] Statistical models 9, 10, and 11 respectively (Table 1).

Means within main effect and canopy columns followed by the same letter are not significantly different (p>0.05, Tukey HSD).

Table 5. Effect of Bt trait, foliar insecticide treatment, or floral structure type on the mean number of injured floral structures in either 2018 and 2019 or 2019 alone

Mean Number of Injured Floral Structures				
Main Effect	**Treatments**	**2018 and 2019**	**2019**	
Trait	Non-Bt	14.04a	11.7a	
	Bt	2.91b	2.49b	
Insecticide	Treated	---	5.01a	
	Untreated	---	5.85a	
Structure	Squares	4.92a	3.83a	
	Bolls	4.21a	2.74a	
	Flowers	0.47b	0.55b	
Type III Tests of Fixed Effects				
Year	**Main Effect**	**F-Value**	**df**	**P-Value**
2018 and 2019[z]	Trait	30.80	1, 3	0.0115
	Structure	14.30	2, 6	0.0052
	Trait*Structure	1.57	2, 6	0.2830
2019[y]	Trait	31.93	1, 2	0.0299
	Insecticide	0.69	1, 2	0.4930
	Structure	34.39	2, 16	<.0001
	Trait*Insecticide	0.12	1, 2	0.7612
	Trait*Structure	0.29	2, 16	0.7490
	Insecticide*Structure	0.40	2, 16	0.6770
	Trait*Insecticide*Structure	0.08	2, 16	0.9203

[z,y] Statistical models 6 and 2 respectively (Table 1).

Means within main effect and year(s) columns followed by the same letter are not significantly different (p>0.05, Tukey HSD).

Table 6. Effect of Bt trait, foliar treatment (lambda-cyhalothrin), or floral structure type on the mean number of *H. zea* larvae in either 2018 and 2019 or 2019 alone

Mean Number of Larvae				
Main Effect	**Treatments**	**2018 and 2019**	**2019**	
Trait	Non-Bt	3.51a	2.43a	
	Bt	0.66b	0.36b	
Insecticide	Treated	---	0.78a	
	Untreated	---	1.08a	
Structure	Squares	0.46b	0.18b	
	Bolls	1.23a	0.65a	
	Flowers	0.23b	0.25b	
Type III Tests of Fixed Effects				
Year	**Main Effect**	**F-Value**	**df**	**P-Value**
2018 and 2019[z]	Trait	15.21	1, 3	0.0299
	Structure	6.39	2, 12	0.0129
	Trait*Structure	1.17	2, 12	0.3436
2019[y]	Trait	28.10	1, 2	0.0338
	Insecticide	0.93	1, 2	0.4361
	Structure	7.36	2, 18	0.0046
	Trait*Insecticide	0.02	1, 2	0.8894
	Trait*Structure	2.54	2, 18	0.1065
	Insecticide*Structure	0.29	2, 18	0.7506

[z,y] Statistical models 8 and 4 respectively (Table 1).

Means within main effect and year(s) columns followed by the same letter are not significantly different (*p*>0.05, Tukey HSD).

DISCUSSION

Non-Bt cotton consistently had more injured structures and larvae in all statistical comparisons, supporting the theory that Bt traits provided some plant protection despite reports of resistance to multiple Bt proteins in the area these studies were performed (Yang et al. 2017). Chlorantraniliprole provided excellent control of *H. zea*, in agreement with wide use of chlorantraniliprole for effective and lasting control of *H. zea* in cotton (e.g., Steckel and Stewart 2012). Moreover, Adams et al. (2016) did not detect meaningful levels of *H. zea* resistance to chlorantraniliprole in the midsouthern United States, while increasing *H. zea* resistance to pyrethroid insecticides has been well documented in the last decade (Musser et al. 2017, Reisig et al. 2019). In fact, treatment with a pyrethroid did not significantly reduce the overall number of injured floral structures or larvae observed in our tests, regardless of Bt or non-Bt variety used. However, the amount of injured structures in the upper portion of the canopy was reduced after treatment with a pyrethroid. The pyrethroid did not significantly affect the number of larvae in the upper canopy, although there was a slight trend indicating a marginal reduction. Presumably, there is better insecticide coverage in the upper canopy, resulting in better larval mortality and a reduction in floral injury. However, it is also possible that the larvae were sub-lethally sickened or had aversion to the pyrethroid insecticide, and thus, the reduction of injury observed in the upper canopy was an anti-feeding response (Hannig et al. 2009). The test in 2018 experienced a rapid onset of bollworm, whereas the tests in 2019 had lower and a slower onset of pest pressure. These sub-threshold infestations were sustained over a period of 14-21 days in 2019, which made it difficult to time a single pyrethroid application and likely contributed to the poor control observed with the pyrethroid insecticide.

The higher proportion of small larvae found in the top portion of the canopy indicates that moths were more likely to oviposit in this portion of the canopy. This finding is not unlike other findings from previous studies (Farrar and Bradley 1985, Gore et al. 2002, Torres and Ruberson 2006). Because samples were collected near physiological cutout, flowers were present in the top portion of the canopy (Bourland et al. 2001). Bollworm moths are attracted to flowers as a source of nectar (Fitt 1989), and thus they may be more likely to oviposit in areas of the canopy where flowers are present (Braswell et al. 2019). Further, the presence of small larvae on small bolls, and especially small bolls with a bloom tag, could be an indicator of oviposition on flowers. In plots that were not treated with an insecticide, across 2018 and 2019, 60.7% of small larvae were found on bolls, and 55.9% of those larvae were found to be on bolls with a bloom tag or small bolls that would have recently shed a bloom. This is substantial given that bolls classified as small bolls or bolls with a bloom tag comprised 30.9% of injured bolls.

The middle portion of the canopy contained a high proportion of medium larvae, which would support previous observations of downward larval movement on cotton plants (Farrar and Bradley 1985, Gore et al. 2002, Braswell et al. 2019). Larvae in early instars feed on squares and begin to feed on bolls after increasing in size (Farrar and Bradley 1985). Bolls in the middle portion of the canopy constitute a sizeable portion of the overall lint yield (Ritchie et al. 2007), thus downward larval movement may have been influenced by preference for, or the high availability of susceptible floral structures. Floral structures in the upper portion of the canopy start decreasing in quantity as the plants mature and larvae feed, thus larvae would be required to move downward to reach more food sources (Braswell et al. 2019). Fewer larvae and injured floral structures were observed in the bottom portion of the canopy. This was likely due to the ovipositional preferences of moths that were previously discussed. At the time of sampling, the bolls in the bottom portion of the canopy would have matured enough to make it difficult for small larvae to successfully establish due to inability to penetrate the boll wall (Benedict et al. 1997).

The distribution of larvae and injury did not significantly differ between different cotton varieties, regardless of the presence of a Bt trait or not. Thus, our results suggest that it would be appropriate to use standardized scouting methods in Bt and non-Bt cotton varieties. Results from Gore et al. (2002) showed that larval behavior in Bollgard (Cry1Ac) cotton may be altered due to the avoidance of high concentrations of Bt proteins. Small but statistically insignificant trends observed in this study suggest the same phenomenon, with a higher proportion of larvae (75% vs. 66%) and injury (65% vs. 59%) occurring lower in the canopy (i.e., middle and bottom portions) of Bt cotton compared with non-Bt cotton. Had we had higher bollworm pressure, this effect may have been more pronounced. Conversely, Jackson et al. (2007) observed no difference in larval movement from the terminal of WideStrike and non-Bt cotton plants over a period of 48 hours, thus providing a precedent example that supports the similarity of injury and larval distribution among non-Bt and Bt cotton. Similarly, this study did not see major effects of pyrethroid treatment on the distribution of larvae or injury to floral structures. Differences may have been more pronounced had a more effective insecticide been used, but pragmatically, this data indicates that changes in the distribution of larvae or injury are not substantial enough to justify different scouting procedures on non-Bt and different Bt cotton varieties or on insecticide treated or non-treated fields. These data would support that scouting efforts could be focused on the middle part of the canopy when cotton is flowering. This study found as many or more small- and medium-sized larvae in the middle portion of the canopy as in the upper canopy. The same pattern was observed with injury. Based on our results and other research, focusing scouting efforts on the middle portion of the canopy should also increase the detection of small larvae and 'fresh' injury and be less influenced by previous insecticide applications.

Not surprisingly, finding injury to floral structures was more common than finding larvae because one larva often feeds on multiple structures (Wilson and Gutierrez 1980). As is in practice today (e.g., Stewart and McClure 2020, Catchot 2020, Ring 2020), treatment thresholds in both non-Bt cotton and Bt cotton are based on larva counts and/or percent injury to floral structures. Given the discussion above, our data suggests sampling of pink flowers and small bolls (including bolls with bloom tags) would be an appropriate scouting method to detect bollworm infestations and make insecticide treatment decisions, at least when bollworm infestations are most likely to occur (at peak flowering and beyond). A recent study on non-Bt and multiple Bt cotton technologies indicated that insecticide management decisions based on injury to squares or small bolls provided economic returns as high or higher than a more proactive and aggressive insecticide approach (Kerns et al. 2017). Insecticide recommendations based on the presence of bollworm eggs does not seem like a sustainable approach where multiple Bt cotton technologies are grown (or non-Bt cotton) because it would require different thresholds based on the efficacy of the technology, which would also be influenced by evolving levels of resistance to Bt toxins (e.g., Tabashnik and Carrière 2017) or difference in expression profiles among plant parts, varieties, or at different times of the season (Sivasupramaniam et al. 2014, Kranthi et al. 2005, Adamczyk et al. 2001, Carrière et al. 2018). While further research is justified, particularly under conditions of very high or at the early onset of bollworm infestation, standardizing insecticide application recommendations for bollworm in non-Bt and Bt cotton varieties may be a simple and appropriate approach.

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