MANAGING STINK BUGS IN *Bt* COTTON

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Abstract

Several species of phytophagous Pentatomidae (*Nezara viridula*, *Acrosternum hilare*, and *Euschistus servus*) were observed as pests on transgenic *Bt* cotton in South Carolina during the 1995-1997 growing seasons. Stink bugs damaged young bolls during mid-to-late season as adults migrated in from alternate hosts. Results from 1995-1997 indicated that control measures for stink bugs produced higher yields than no control. Treating with methyl parathion at a threshold of one stink bug per six feet of row provided adequate protection of developing bolls. Use of a ground-cloth is recommended in order to determine stink bug density per linear foot of row. We recommend insecticide treatment at a density of one bug per six feet of row. Careful attention should also be given to the percentage of young bolls damaged, and our data indicated that treatment might be necessary if more than 20% of bolls are penetrated in mid-to-late season. Bolls aged 18 days from white bloom were not significantly damaged by late instars (4th and 5th) which caused more damage than all other life stages.

Introduction

In South Carolina, several phytophagous pentatomids (*Nezara viridula*, *Acrosternum hilare*, and *Euschistus servus*) are generally considered secondary pests in conventional cotton cultivars. Many growers do not monitor and treat for stink bugs in conventional systems because of fortuitous suppression/control of bugs with mid-to-late-season pyrethroid applications for our major pest the cotton bollworm, *Helicoverpa zea* (Sullivan et al. 1996). Others might apply one or two applications of methyl parathion late in the season regardless of bug density.

Reduced pyrethroid use in transgenic *Bacillus thuringiensis* (*Bt*) cultivars allows plant-feeding stink bugs to colonize *Bt* fields during critical periods of boll development, and these reproducing populations can cause significant reductions in yield and fiber quality (Wene and Sheets 1964, Toscano and Stern 1976, Roach 1988, Barbour et al. 1990, Turnipseed et al. 1995, Turnipseed and Greene 1996). The potential for crop losses by stink bugs and the lack of information about their management in cotton prompted us to begin developing treatment thresholds for stink bugs in *Bt* cotton (Greene et al. 1996). This paper summarizes three years of research.

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Materials and Methods

Threshold Plots

Cotton was grown using recommended production practices. Site 1 (Sandifer Farm) located near Blackville, SC was planted 8 May in 1995, 6 May in 1996, and 13 May 1997. Site 1 was bordered by oaks, other hardwoods, pines, blackberries, and additional cotton. Site 2 (Edisto Research and Education Center [EREC]) located near Blackville, SC was planted 3 May 1996 and 25 April 1997 and bordered by non-cultivated areas, southern peas, and additional cotton. Two Delta Pine (DP) varieties were used: (1) *Bt* (Bollgard™) NuCOTN33b and (2) the conventional parent DP5415. Large field plots of 24 rows by 80 feet (0.14 acre) were used in a randomized complete block design with four replications. In 1995, at least 12 rows of non-*Bt* DP5415 border cotton planted on the sides of experimental plots and 40 feet planted at the plot ends remained untreated. Border rows were not used in 1996 or 1997. Treatment thresholds for 1995 and 1996 (Greene et al. 1997) were modified for the 1997 season because: we seldom reached a threshold of one bug per three feet of row, and we wanted to compare control with pyrethroids and methyl parathion.

	Threshold Level	
Treatment	Stink Bug	Bollworm
1) *Bt* NuCOTN33b	1 bug/12' w/m.p.	untreated
2) *Bt* NuCOTN33b	1 bug/6' w/m.p.	untreated
3) *Bt* NuCOTN33b	1 bug/6' w/pyreth	untreated
4) *Bt* NuCOTN33b	untreated	untreated
5) *Bt* NuCOTN33b	1 bug/6' w/m.p.	pyreth@peak egg lay
6) Non-*Bt* DP5415	1 bug/6' w/m.p.	pyreth@3worms/100
7) Non-*Bt* DP5415	untreated	untreated

Weekly sampling, using dishpan or ground-cloth methods as described by Greene and Turnipseed (1996), was initiated in July to assess populations of stink bugs, predaceous arthropods, and other insects. On each sampling date, five ground-cloth samples were taken randomly from the 12 middle rows in each plot, and numbers of nymphs and adults were recorded for green, southern green, and brown stink bugs. After a stink bug threshold was reached using an average from all plots in a treatment, methyl parathion (4EC) at 0.50 lb (AI)/acre or Karate® (1EC) at 0.033 lb (AI)/acre (treatment #3) was applied with a high-clearance sprayer to control populations.

Numbers of bollworm eggs and larvae were observed and recorded biweekly on the top third of 25 plants per plot with larvae being classified as small (< 0.25 inch) or medium-large ($\geq$ 0.25 inch). Karate® 1EC (cyhalothrin) at 0.033 lb (AI)/acre or Baythroid® 2 (cyfluthrin) at 0.05 lb (AI)/acre were applied with a high-clearance sprayer after reaching threshold levels of bollworm in non-*Bt* cotton. In 1997, one treatment included *Bt* cotton treated with pyrethroid 2-5 days following peak bollworm oviposition and again when medium-large bollworm numbers exceeded 4 larvae per 100 plants.

Percentages of boll damage by stink bugs were determined on sampling dates by randomly collecting and opening 25 bolls (50 to 75% grown) from the 12 center rows of each plot for a total of 100 bolls per treatment. Each boll was squeezed open by hand and considered damaged if at least one puncture wound/wart was observed on the interior wall.

Seed cotton yields were determined by mechanically harvesting the four middle rows using a two-row picker or by manually harvesting 10 ft sections of row in each plot (Site 2 1997). Yields are reported as pounds of seed cotton per acre. All data were subjected to ANOVA using PROC GLM, and treatment means were separated ($P \leq 0.05$) using Least Significant Difference (LSD) (SAS Institute 1985, Badarinathi 1992).

Cage Studies

Large, walk-in, insect cages (Lumite® screen by Synthetic Industries) measuring 6 ft high, 6 ft wide, and 12 ft long were assembled with PVC frames and placed in a *Bt* cotton field at EREC on 16 July 1997. Each of the four cages covered two 12 ft sections of row and prevented undesired insect damage to developing reproductive structures. On 17 and 18 July, Karate® 1EC at 0.04 lb AI/acre was applied to cotton inside the cages to kill arthropods therein. White blooms inside the four screen cages were tagged biweekly with flagging tape and dated.

On 23 July, 120 small cages made of 12 oz Styrofoam cups with bottom removed and pantyhose were placed over bolls 8 days old, which were previously tagged as blooms, and secured with rubber bands. On 24 July in an experiment to determine the extent of boll damage caused by various life stages of stink bugs, stink bugs (second, third, fourth, and fifth instars and adults) from a laboratory-reared colony of *Nezara viridula* were placed singly inside the small cup cages in a completely randomized design with 20 replications per treatment. Twenty tagged bolls were also caged without bugs as checks. Bugs were housed in the cups for 3.5 days before removal from the cup cages. In all cage experiments, bolls were examined individually four days after bug removal for feeding damage by counting wart-like growths on interior carpel walls. A completely destroyed boll was given the value of 30 warts, since this number approximated the maximum number of countable warts inside a severely damaged boll.

In a second experiment beginning on 5 August, we examined the effect of boll age on stink bug feeding and damage. Late fourth or early fifth instars were assigned (in a completely randomized design with 20 replications per treatment) singly to cup cages placed on bolls aged (from white bloom) 4, 8, 15, and 18 days. Five bolls from each age group were caged without bugs. Bugs were housed for 5 days before removal from the cup cages.

On 6 August, a third experiment addressed whether or not tarnished plant bug, *Lygus lineolaris*, caused boll damage similar to damage by stink bugs. Late fourth instar *N. viridula* and adult plant bugs were confined (in a completely randomized design with 10 replications per treatment) to a single boll in cup cages placed on 8-day-old bolls. Ten bolls were caged without bugs. Bugs were housed for 10 days before removal from the cups.

In a fourth experiment beginning on 15 August, we investigated boll damage by fifth instar *N. viridula* and corresponding yield reduction. Fifth instar *N. viridula* were confined (in a completely randomized design with 20 replications per treatment) in cup cages over 13-day-old bolls. Twenty bolls were caged without bugs as a check. Bugs were housed for 7 days before removal from the cups. Half of the bolls were examined for warts 4 days after bug removal, and the other half was allowed to mature for yield determination.

Results and Discussion

Threshold Plots

For control of budworm/bollworm on non-*Bt* cotton (DP5415), pyrethroid insecticide was applied eight, four, and five times in 1995, 1996, and 1997, respectively (Tables 1-3). Due to combined stink bug and bollworm damage, untreated non-*Bt* plots produced significantly less seed cotton than all other treatments (Tables 1 and 3), except for untreated *Bt* cotton at Site 1 in 1996 (Table 2). Transgenic *Bt* cotton treated two or four times with pyrethroid insecticide for budworm/bollworm produced an average of 699 lbs more seed cotton than untreated *Bt* cotton, indicating that supplemental control for *H. zea* and stink bugs was necessary (Tables 2 and 3).

Stink bugs were the major secondary pests encountered. The predominant species were the southern green stink bug, *N. viridula*, and the green stink bug, *A. hilare*. Applications of methyl parathion reduced boll damage by stink bugs, and pyrethroid use also offered suppression of bug damage (Tables 1-3). Turnipseed et al. (1995) also found that stink bug damage to developing bolls was directly related to pyrethroid applications.

Seed cotton yields from *Bt* cotton treated with one to three applications of methyl parathion did not differ significantly (Tables 1-3). Transgenic *Bt* plots treated with methyl parathion at one stink bug per six feet of row generally produced higher yields than *Bt* cotton treated at one bug per three feet of row.

Cage Studies

Results concerning boll damage caused by different life stages of stink bugs indicated that boll damage increased with nymphal stage, and 5th instar *N. viridula* caused significantly more damage to 9-day-old bolls than all other life stages (Figure 1). Over the 3.5 day caging/feeding period, damage by individual 2nd instars did not differ significantly from the check group. During the first experiment, several female adults produced egg masses inside the cup cages, and parasitoids (*Trichopoda pennipes*, a tachinid parasitoid of adult *N. viridula*) emerged from two field-collected adults. Stink bug oviposition and adult parasitism contributed to a reduction in average boll damage by adults (Figure 1).

Results concerning the effects of boll age on stink bug feeding and damage indicated that boll damage by late 4th or early 5th instars decreased with increasing boll age (Figure 2). Bolls aged 4-15 days from white bloom were damaged significantly during a 5-day feeding period. Bolls aged 18 days from white bloom were not significantly damaged when compared to the check group (Figure 2).

Results concerning whether or not tarnished plant bug, *L. lineolaris*, caused boll damage similar to damage by stink bugs indicated that adult plant bugs and 5th instar stink bugs, *N. viridula*, both caused significant damage to 8-day-old bolls over a 10-day feeding period. Adult plant bugs caused significantly less damage than larger stink bug nymphs, but the internal damage closely resembled that of stink bug damage. Plant bugs usually prefer to feed on squares, but when forced for a long period of time (10 days) will feed on and damage very small bolls (Figure 3). Squares are available during young boll development, so boll damage by plant bugs should not be a significant problem.

Results concerning boll damage by *N. viridula* and corresponding yield reduction indicated that stink bug 5th instars caged for 7 days on 13-day-old bolls examined for damage resulted in the development of approximately 8.5 warts per boll (Figure 4). For bolls taken to yield (Figure 4), a 54% reduction in yield was observed under identical insect pressure.

Summary

These results demonstrate that stink bugs can cause significant damage to untreated *Bt* cotton, and even with supplemental bollworm control, additional stink bug control may be necessary. Large instars (4th and 5th) caused significant damage to developing bolls (Figure 1), but all stages except non-feeding 1st instars should be included in threshold counts, as early instar development is rapid and adults can also cause significant damage. Proper management of stink bugs in *Bt* cotton should begin with careful ground-cloth sampling in mid-season. Our three year study indicated that insecticide treatment at the density of one bug per six feet of row resulted in the highest seed cotton yield of stink bug thresholds tested (3141 lbs/acre). At this threshold, two applications of methyl parathion were usually necessary during August to protect developing bolls from stink bug damage. Methyl parathion is an effective and economical material for stink bugs, but pyrethroids are effective when problems with both stink bugs and bollworm are encountered.

Bolls from field plots were considered damaged if at least one wart was observed on the inner boll walls, and considerable time was saved during boll examinations by using this method of damage determination. Our data indicated that approximately 20% boll damage was observed at the density of one bug per six feet of row. Plant bugs might damage young bolls during July (Figure 3), but square availability should preclude this. Bolls aged less than 18 days from white bloom remained vulnerable to feeding and damage by stink bugs.

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Table 1. Percent boll damage by stink bugs and seed cotton yields in treated and untreated *Bt* (NuCOTN 33) and non-*Bt* (DPL 5415) cotton. Site 1 (Sandifer) in Blackville, SC (1995).

Treatment	**% Damaged[a,b]** 29 Aug.	8 Sept.	22 Sept.	**Seed Cotton (lbs / acre)[a]**
B.t. 1bug/ 12 ft	[m] 11 b	33 b	[m] 35 bc [m]	2566 a
B.t. 1 bug/ 6 ft	[m] 16 ab	38 ab	[m] 32 c	2588 a
B.t. 1 bug/ 3 ft	31 a	50 ab	[m] 25 c	2463 a
B.t. untreated	26 ab	38 ab	65 ab	1844 b
5415 pyrethroid	[ppp] 12 b	[p] 8 c	[p] 9 c	2468 a
5415 untreated	32 a	58 a	77 a	662 c

[a] Treatment means within a column followed by the same letter do not significantly differ (α = 0.05), LSD.
[b] Sample proportions presented. Analyses performed on arsine-transformed data.
[m] Methyl parathion 4EC application at 0.50 lb (AI)/acre between sampling dates.
[p] Pyrethroid application (Karate 1EC® at 0.033 lb [AI]/acre) between sampling dates.

Table 2. Percent boll damage by stink bugs and seed cotton yields in treated and untreated *Bt* (NuCOTN 33) and non-*Bt* (DPL 5415) cotton. Site 1 (Sandifer) in Blackville, SC (1996).

Treatment	**% Damaged[a,b]** 24 July	6 Aug.	20 Aug.	**Seed Cotton (lbs / acre)[a]**
B.t. 1bug/ 12 ft	16 ab	[m] 27 ab	[mm] 10 bcd	3523 bc
B.t. 1 bug/ 6 ft	13 ab	[m] 20 ab	[m] 9 cd	3904 ab
B.t. 1 bug/ 3 ft	15 ab	30 ab	[m] 25 abc	3665 ab
B.t. untreated	16 ab	34 ab	32 ab	3052 cd
B.t. pyrethroid	[p] 10 b	[pp] 8 c	[p] 1 d	4065 a
5415 pyrethroid	[p] 14 ab	[pp] 8 c	[p] 2 d	3830 ab
5415 untreated	25 a	36 a	38 a	2655 d

[a] Treatment means within a column followed by the same letter do not significantly differ (α = 0.05), LSD.
[b] Sample proportions presented. Analyses performed on arsine-transformed data.
[m] Methyl parathion 4EC application at 0.50 lb (AI)/acre between sampling dates.
[p] Pyrethroid application (Baythroid 2® at 0.05 lb [AI]/acre) between sampling dates.

Table 3. Percent boll damage by stink bugs and seed cotton yields in treated and untreated *Bt* (NuCOTN 33) and non-*Bt* (DPL 5415) cotton. Site 2 (EREC) in Blackville, SC (1997).

Treatment	**% Damaged[a,b]** 24 July	6 Aug.	20 Aug.	**Seed Cotton (lbs / acre)[a]**
B.t. 1bug/ 12 ft	32 a	[m] 10 ab	[m] 10 cd	2834 a
B.t. 1 bug/ 6 ft	34 a	22 a	[m] 29 ab [m]	2930 a
B.t. 1 bug/ 6 ft	35 a	20 a	[p] 11 cd	2820 a
B.t. untreated	30 a	20 a	23 bc	2504 a
B.t. pyrethroid	29 a	[p] 12 ab	[p] 7 d	2889 a
5415 pyrethroid	[pp] 9 b	[p] 1 b	[pp] 4 d	2696 a
5415 untreated	28 a	22 a	36 a	1018 b

[a] Treatment means within a column followed by the same letter do not significantly differ (α = 0.05), LSD.
[b] Sample proportions presented. Analyses performed on arsine-transformed data.
[m] Methyl parathion 4EC application at 0.50 lb (AI)/acre between sampling dates.
[p] Pyrethroid application (Karate 1EC® at 0.033 lb [AI]/acre) between sampling dates.