# INFLUENCE OF REFUGIA ON MOVEMENT AND DISTRIBUTION OF BOLLWORM/TOBACCO BUDWORM LARVAE IN BOLLGARD COTTON

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## Abstract

A field study was conducted to compare the relative densities, distribution patterns, and economic impact of bollworms, Helicoverpa zea (Boddie), and tobacco budworms, Heliothis virescens (F.), in Monsanto's transgenic cotton 'BT01DP' expressing the delta-endotoxin from the bacterium, Bacillus thuringiensis subsp. kurstaki (Berliner) (*Bt*), a 90:10 *Bt*:non-*Bt* seed mix, and nontransgenic 'Coker 312' cotton. These treatments received no applications of insecticides for control of lepidopteran insects. The bollworm was the predominant species, comprising approximately 95% of the bollworm/tobacco budworm complex. Examination of ten consecutive plants in each of the two center rows of each plot revealed that the 16.7% of plants in the 90:10 *Bt*:non-*Bt* plots infested with third-instar and larger larvae was significantly greater than the expected percentage of 9.5. Similarly, the 9.2% of plants in the 90:10 plots infested with fourth-instar and larger larvae was significantly greater than the expected percentage of 4.9. These higher than expected percentages of plants infested with large larvae in the 90:10 Bt:non-*Bt* plots indicated that movement of larvae from non-*Bt* to *Bt* plants occurred. Results indicated that movement of bollworm larvae to *Bt* plants may result in a significant increase in damage and reduced yield in mixed stands of *Bt* and non-*Bt* plants. Additional studies are needed to determine the relationships which exist among seed mix ratios, population densities of bollworms/tobacco budworms, injury, and yield reductions.

## Introduction

The bollworm, Helicoverpa zea (Boddie),/tobacco budworm, Heliothis virescens (F.), complex is the most serious insect pest of cotton in South Carolina. In 1994, this pest complex exceeded treatment thresholds in 97% of the state's cotton acreage and caused a 3.4% reduction in yield despite the application of 4.4 insecticide treatments (Williams 1995). Transgenic (*Bt*) cotton expressing the insecticidal protein, delta-endotoxin, from Bacillus thuringiensis subsp. kurstaki (Berliner) is extremely effective against bollworms and tobacco budworms in South Carolina, greatly reducing but not completely eliminating damage by these pests (DuRant 1994, 1995). Mahaffey et al. (1994, 1995) reported that unusually high population densities of the bollworm, known to be less sensitive to *Bt* toxins than the tobacco budworm (Stone and Sims 1993), caused significant levels of damage to fruit of *Bt* cotton in North Carolina.

Although Monsanto Company will market 100% *Bt* cotton seed initially, seed mixes containing a small percentage of non-*Bt* seed remain a possible future alternative strategy for resistance management. The non-*Bt* plants would provide a refuge for susceptible insects within the population, thus maintaining a high frequency of susceptible genes within that population (Fischoff 1992).

A major concern accompanying the use of seed mixes of *Bt*/non-*Bt* cotton is the potential increase in damage to fruit and resulting reductions in yield caused by bollworm/tobacco budworm larvae which originate on the non-*Bt* plants and subsequently move to *Bt* plants. Parker and Luttrell (1995) concluded that tobacco budworm larvae surviving on non-*Bt* cotton up to 8 days potentially could move to a *Bt* cotton plant and damage the fruit. Halcomb et al. (1994) investigated the instar specific survival of bollworm and tobacco budworm larvae on mixtures of *Bt* and non-*Bt* cotton and found that fifth-instar larvae of both species placed on *Bt* flower buds survived to the adult stage. Survival was greater for bollworms (46.6%) than for tobacco budworms (27.1%). They concluded that additional studies under field conditions are needed to quantify the relationships among bollworm/tobacco budworm injury, yields, and seed mix ratios.

The objective of this study was to determine the relative densities, distribution patterns, and economic impact of bollworms and tobacco budworms in 100% *Bt* cotton, 90% *Bt* cotton, and non-*Bt* cotton.

## Materials and Methods

Cotton seed containing the Bollgard gene having a germplasm background of 'Coker 312' and designated 'BT01DP' and non-transgenic 'Coker 312' seed were provided pre-mixed by Monsanto Company, St. Louis, MO, in *Bt*:non-*Bt* ratios of 100:0, 90:10, and 0:100. Plots were planted at the Pee Dee Research and Education Center in Darlington County, SC, on 3 May, 1995, in Wagram sand soil. Aldicarb (Temik 15G) was applied in-furrow at 0.83 lb(AI)/acre. Row and plant spacings were 38 and 3.5 in., respectively. On 14-15 June, plant stands were thinned to 2 plants/ft. Recommended agronomic practices were followed. Six replications of each treatment (seed mix) were arranged in a randomized complete-block design and plots were 8 rows by 30 ft. Ten consecutive plants in each of the two center rows of each plot were tagged and these plants were examined on 13, 20, 27 July and 3, 10 August. All white blooms, damaged and undamaged bolls and squares, and bollworm/tobacco budworm eggs and larvae were recorded for each plant. Only economically damaged squares and bolls, i.e., squares that will abort due to feeding through the calyx or corolla to the anthers and bolls that will sustain loss of one or more locules due to feeding through the carpel wall to the seed, were considered damaged. The instar and location of each larva was recorded. Standard scouting procedures were used throughout the season to monitor the plots for infestations of pests other than bollworms and tobacco budworms. Larvae were collected from untreated cotton cultivar 'DPL 5415' adjacent to the test plots on 25-28 July (50 larvae) and 4 August (54 larvae) for species determination. On 17 August, 41 larvae were collected from bolls in adjacent plots of BT01DP:non-*Bt* seed mixes ranging from 100:0 to 0:100. On 18 August, 28 larvae were collected from adjacent Bollgard cotton (100% *Bt*). Plots were defoliated on 9 October using Folex 6EC (1.5 pt/acre). Yields were determined by hand harvesting each of the 360 tagged plants on 27-28 September, and by machine harvesting the four center rows of each plot on 6 November. Lint percentages were determined by ginning the hand-harvested seed cotton and ca. 1 lb of the machine harvested seed cotton from each plot using a 10-saw laboratory gin. Data were subjected to analysis of variance and means were separated using Fisher's protected LSD test ($P = 0.05$) (Gylling and Gylling 1992). Data on fruit damage and population densities of larvae were transformed to square root($x + 0.5$) for analysis, but original means are presented. Chi-square analysis was used to determine the significance of the differences between expected and observed values for the 90:10 *Bt*:non-*Bt* mix for numbers of plants with damaged bolls and numbers of plants with third-instar and larger larvae and fourth-instar and larger larvae. Expected values were calculated as weighted means derived from observed values for 100% *Bt* cotton and non-*Bt* cotton, assuming that no inter-plant movement of larvae occurred.

## Results and Discussion

The species composition of 50 larvae collected from untreated cotton on 25-28 July was 74% bollworms, 16% bollworms/tobacco budworms (first and second instars which were not identified), 4% tobacco budworms, and 6% fall armyworms, Spodoptera frugiperda (J.E. Smith). Fifty-four larvae collected on 4 August were 81% bollworms, 9% bollworms/tobacco budworms, 4% tobacco budworms, and 6% fall armyworms. The 41 larvae collected from bolls on 17 August were 78% bollworms, 2% tobacco budworms, and 20% fall armyworms. The 28 larvae collected from Bollgard cotton on 18 August were 36% bollworms, 36% fall armyworms, and 28% beet armyworms, S. exigua

(Hubner). On 28 July and 4 August the test was treated with dicrotophos (Bidrin 8) at 0.50 lb(AI)/acre to control a moderate infestation of green stink bugs, Acrosternum hilare (Say).

Densities of eggs and larvae of the second generation of bollworms/tobacco budworms remained unusually low during June and never reached economic levels. Oviposition by moths of the second generation was initiated during the second week of July and peaked in late July (Table 1). Although densities of eggs were not affected significantly by treatment (seed mix) except for 20 July, slightly fewer eggs were observed in the 100% *Bt* plots compared with the 90:10 and non-Bt plots for all dates except 13 July. Densities of squares, white blooms, and bolls were not altered significantly by treatment for any of the five observation dates, indicating that the fruiting characteristics were essentially the same for the Bollgard and non-transgenic plants. Densities of larvae, which ranged from 1/100 plants for 100% *Bt* cotton on 20 July to 30/100 plants for non-transgenic cotton on 10 August, were not altered significantly by treatment except for 3 and 10 August, when significantly greater numbers of larvae occurred in the non-transgenic plots compared with the 100% *Bt* plots (Table 2). Many of these larvae were early instars which likely failed to survive. Table 3, which includes only larger larvae (third instar and above), presents a more realistic assessment of the efficacy of the 90:10 seed mix, which compared favorably with the 100% *Bt* cotton until 10 August, when densities of large larvae were ca. three times the densities for the 100% *Bt* cotton and half the densities for the non-*Bt* cotton. Damage to squares was relatively low, remaining below 5% except for the non-*Bt* cotton on 10 August (Table 4). Damage to bolls also was low throughout the season, but was ca. 100% greater in the 90:10 plots than in the 100% *Bt* plots (Table 5).

The percentage of plants in the 90:10 plots with third-instar and larger larvae was 16.7, an increase of 75.8% over the expected percentage of 9.5 (Table 6). Similarly, the percentage of plants with fourth-instar and larger larvae was 9.2, an increase of 87.8% over the expected percentage of 4.9. Chi-square analysis indicated that these differences were significant ($P = 0.05$). Although the percentage of plants with damaged bolls was 30.9, an increase of 52.2% over the expected percentage of 20.3, this difference was nonsignificant.

Lint percentages for tagged plants which were harvested by hand on 27-28 September ranged from 38.7 for the 90:10 plots to 40.4 for the nontransgenic plots, and differences among treatments were nonsignificant (Table 7). Lint percentages for the machine-harvested rows on 6 November, however, ranged from 38.2 for the nontransgenic plots to 40.3 for the 100% *Bt* plots, and were significantly greater for the 100% *Bt* plots and the 90:10 plots than for the nontransgenic plots. These differences in lint percentages between the hand-harvested and machine-harvested samples likely were due to the different methods of harvesting. Also, the four center rows yielded a more representative sample than did the 20 tagged plants. Yields of lint were relatively low, ranging from 256.8 - 291.1 lb/acre for the nontransgenic and 90:10 plots, respectively, for the hand-harvested plants. Similarly, yields for the machine-harvested rows ranged from 249.2 - 301.0 lb/acre for the nontransgenic and 100% *Bt* plots, respectively. Differences among yields were nonsignificant for both methods of harvesting.

## Summary and Conclusions

The bollworm was the predominant species in this study. Tobacco budworms comprised less than 5% of the bollworm/tobacco budworm complex. Although fall armyworms became more abundant during late August, they comprised only 6% of the population of larvae during late July and early August. Hence, we are confident that the data on damage to cotton recorded during the study period are predominantly for the bollworm. The higher than expected percentages of plants infested with large larvae in the 90:10 *Bt*:non-*Bt* plots indicated that movement of larvae from non-*Bt* to *Bt* plants occurred, resulting in increased damage to bolls. Results of this study indicate that movement of larvae from non-*Bt* to *Bt* plants may result in significant increases in damage and reduced yields in mixed stands of *Bt* and non-*Bt* plants. Additional studies are needed to determine the relationships which exist among seed mix ratios, population densities of bollworms/tobacco budworms, injury, and yield reductions.

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Table 1. Effect of treatment (seed mix) on densities of bollworm eggs. Darlington County, SC, 1995.

| ***Bt*:non-*Bt*** | **Eggs/100 plants** | | | | | |
|---|---|---|---|---|---|---|
| | **July** | | | **August** | | **Seasonal** |
| **ratio** | **13** | **20** | **27** | **3** | **10** | **mean** |
| 100:0 | 10a | 12b | 23a | 4a | 6a | 10.5a |
| 90:10 | 10a | 32a | 29a | 8a | 18a | 19.4a |
| 0:100 | 10a | 24a | 34a | 5a | 9a | 16.7a |

Means within a column followed by the same letter are not significantly different (P = 0.05, Fisher's protected LSD test [Gylling and Gylling 1992]).

Table 2. Effect of treatment (seed mix) on densities of bollworm larvae. Darlington County, SC, 1995.

| *Bt*:non-*Bt* | Larvae/100 plants | | | |
|---|---|---|---|---|
| ratio | 20 July | 27 July | 3 August | 10 August |
| 100:0 | 1.0a | 5.0a | 8.5b | 6.5b |
| 90:10 | 5.0a | 7.5a | 16.5ab | 16.0ab |
| 0:100 | 8.5a | 13.5a | 26.0a | 30.0a |

Means within a column followed by the same letter are not significantly different (P = 0.05, Fisher's protected LSD test [Gylling and Gylling 1992]). Square root(x + 0.5) transformation, original means presented.

Table 3. Effect of treatment (seed mix) on densities of large bollworm larvae. Darlington County, SC, 1995.

| *Bt*:non-*Bt* | Larvae/100 plants[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ratio | 20 July | | 27 July | | 3 August | | 10 August | |
| | 3+ | 4+ | 3+ | 4+ | 3+ | 4+ | 3+ | 4+ |
| 100:0 | 0.0a | 0.0a | 1.0a | 1.0ab | 3.5b | 1.0a | 4.0b | 2.5b |
| 90:10 | 1.5a | 1.0a | 1.0a | 0.0b | 5.0b | 1.5a | 12.5b | 7.5ab |
| 0:100 | 2.5a | 1.0a | 6.0a | 5.0a | 13.5a | 6.5a | 23.5a | 15.0a |

Means within a column followed by the same letter are not significantly different (P = 0.05, Fisher's protected LSD test [Gylling and Gylling 1992]). Square root(x + 0.5) transformation, original means presented.

[a] 3+, third instar and larger; 4+, fourth instar and larger.

Table 4. Effect of treatment (seed mix) on damage to squares by bollworms. Darlington County, SC, 1995.

| *Bt*:non-*Bt* | % Damaged squares | | | | | |
|---|---|---|---|---|---|---|
| | July | | | August | | Seasonal |
| ratio | 13 | 20 | 27 | 3 | 10 | mean |
| 100:0 | 0.1a | 0.2a | 0.3a | 2.4a | 3.7ab | 1.34b |
| 90:10 | 0.1a | 0.7a | 0.9a | 0.2a | 0.0b | 0.37b |
| 0:100 | 0.6a | 0.8a | 1.4a | 4.3a | 15.2a | 4.43a |

Means within a column followed by the same letter are not significantly different (P = 0.05, Fisher's protected LSD test [Gylling and Gylling 1992]). Square root(x + 0.5) transformation, original means presented.

Table 5. Effect of treatment (seed mix) on damage to bolls by bollworms. Darlington County, SC, 1995.

| *Bt*:non-*Bt* | % Damaged bolls | | | | |
|---|---|---|---|---|---|
| | July | | August | | Seasonal |
| ratio | 20 | 27 | 3 | 10 | mean |
| 100:0 | 0.0a | 0.1b | 1.0b | 2.1b | 0.80b |
| 90:10 | 0.8a | 0.4b | 1.7b | 4.1b | 1.74b |
| 0:100 | 0.9a | 2.0a | 4.6a | 8.3a | 3.98a |

Means within a column followed by the same letter are not significantly different (P = 0.05, Fisher's protected LSD test [Gylling and Gylling 1992]). Square root(x + 0.5) transformation, original means presented.

Table 6. Observed versus expected percentages of plants infested with bollworms/tobacco budworms and percentages of plants with damage to bolls, for the 90:10 *Bt*:non-*Bt* plots. Darlington County, SC, 1995.

| | % Plants | |
|---|---|---|
| | Observed | Expected[a] |
| 3rd instar larvae and larger[b] | 16.7 | 9.5* |
| 4th instar larvae and larger[c] | 9.2 | 4.9* |
| Damaged bolls[d] | 30.9 | 20.3 |

[a]Calculated as weighted means derived from observed values for 100% *Bt* cotton and non-*Bt* cotton. Values followed by an asterisk differ significantly (P = 0.05, chi-square analysis) from the observed values.

[b] Observed values were 6.5 for 100% *Bt* cotton, 36.0 for non-*Bt* cotton.

[c] Observed values were 2.5 for 100% *Bt* cotton, 26.5 for non-*Bt* cotton.
[d] Observed values were 16.5 for 100% *Bt* cotton, 54.0 for non-*Bt* cotton.

Table 7. Effect of treatment (seed mix) on yield. Darlington County, SC, 1995.

| ***Bt*:non-*Bt*** | **27 September** | | **6 November** | |
|---|---|---|---|---|
| | **%** | **Lb lint/** | **%** | **Lb lint/** |
| **ratio** | **Lint** | **acre** | **Lint** | **acre** |
| 100:0 | 39.4 a | 261.5 a | 40.3 a | 301.0 a |
| 90:10 | 38.7 a | 291.1 a | 39.3 a | 288.3 a |
| 0:100 | 40.4 a | 256.8 a | 38.2 b | 249.2 a |

Means within a column followed by the same letter are not significantly different (P = 0.05, Fisher's protected LSD test [Gylling and Gylling 1992]).