Distribution of Heliothine Larvae in *Bt* and Non-*Bt* Cotton in Texas

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ABSTRACT

Knowledge of population dynamics in heliothines in transgenic cotton is needed to develop resistance management strategy and scouting guidelines. The spatial distribution of heliothine larvae within and among transgenic cotton plants is likely to be influenced by variation in toxin expression within individual plants and throughout the growing season. A study was conducted to assess larval survival among diverse cotton plant structures to determine whether variation in larval survival was a function of the proportion of resistant individuals in the population or due to sub-lethal levels of toxin expression in various plant tissues. The study was motivated by Bt cotton failure in Brazos River Bottom in 1996 when Helicoverpa zea larvae were found in flowers, apparently feeding on pollen with low toxin content. In 1997, Bt cotton cultivars carrying the Cry1Ac toxin gene and non-Bt cotton were scouted in pairs in four locations in the Brazos River Bottom around College Station, Texas. The total effort was 79.5 man-hours for non-Bt cotton and 64.75 man-hours for Bt cotton. A total of 2875 plants were scouted in non Bt cotton and 2825 in Bt cotton. On each scouting day, 100 plants were selected randomly and scouted thoroughly. Larvae were classified as found on terminals, squares, flowers and bolls. The node position was also recorded. The data indicate that the majority of the larvae are found in terminals and squares in Bt cotton and that the terminal is a critical factor in the mortality of heliothine larvae exposed to Bt cotton. Data for 1998 show a similar trend. Third instar larvae found in flowers in Bt cotton were identified as H. zea or Spodoptera spp., as expected. The project was funded by a grant from Cotton Inc. to the primary author.

Introduction

Knowledge of population dynamics of heliothines in transgenic cotton is needed to develop resistance management plans and scouting guidelines: the larval distribution of heliothines in transgenic *Bacillus thuringiensis (Bt)* cotton expressing the Cry1Ac toxin has not been previously reported. In addition, the vertical distribution of larvae within cotton plants and their distribution among different plant structures may reflect changes in the level of *Bt* toxin available in diverse plant tissues (McGaughey and Whalon, 1992). These toxin levels may vary not only within a single plant but also between plants at a specific time, or suffer an overall decrease throughout the growing season (Greenplate, 1999). If the toxin levels in certain plant structures provide a sublethal toxin dose for a certain proportion of larvae, the refuge strategy for resistance management may be in jeopardy (Gould, 1998). This is so because the refuge strategy is based on the assumption that the heterozygote larvae for Cry1Ac toxin-resistance will be killed within the *Bt* cotton canopy (Gould, 1998). Survival of larvae in diverse plant structures may alternatively indicate the presence of Cry1Ac resistant individuals. Laboratory detection of resistant larvae of *Helicoverpa zea* is complicated by the fact that the natural tolerance of this species is highly variable (Sims *et al.*, 1993), in contrast, the Cry1Ac toxin exhibits a high potency for *Heliothis virescens* (Luttrell *et al.*, 1999).

This study was motivated by apparent failures in the Brazos River Bottom in 1996, when third instar and greater *H. zea* larvae were found in flowers, apparently feeding on pollen with low toxin content (Fox, 1997; Greenplate, 1997). We investigated the spatial distribution of Heliothine larvae in *Bt* cotton during the second and third year after the introduction of commercial *Bt* cotton in the Brazos River Bottom in Texas. The purpose of this study was to:

1) Measure the number of larvae present in diverse plant structures immediately after the commercial adoption of *Bt* cotton cultivars; therefore under conditions in which the presence of homozygote resistant individuals (rr) is highly unlikely. When possible, identify the species of target individuals (*Heliothis* vs. *Helicoverpa*) that survive on these plant structures. This measurement will permit identification of those plant structures that are effective in killing heliothine larvae or executing a "high dose strategy" in accordance to the *Bt* cotton/ refuge plan.

2) Compare the vertical distributions of heliothine larvae in conventional and *Bt* cottons to develop effective and efficient sampling plans. This information is also needed as a reference to detect changes in the future that may reflect the presence of resistant individuals.

Understanding the heliothine survivorship in these two production schemes will assist in determining the appropriate sizes of refuges.

Materials and methods

Scouting and fields: In 1997, fields with cotton varieties carrying the Bollgard™ gene (Cry1Ac toxin) and conventional non- *Bt* cotton were scouted in pairs in four locations in the Brazos River Bottom around College Station, Texas. In all, 2,875 plants were scouted in *non-Bt* cotton and 2,825 plants in *Bt* cotton. On each scouting day, 100 plants were selected randomly and each plant was scouted thoroughly from the terminal to the first node, including leaves and all reproductive tissues. The locations (by node and by plant structure) and developmental stages of all heliothine larvae found on sample plants were recorded. Plant structures were divided into terminals, squares, flowers (including tags) and bolls. The four field pairs were designated as N, P, USDA/AM, and H, respectively. Three of the *Bt* cotton fields (P, N, AM) were commercially managed and received pesticide applications; the fourth (H) was not treated with pesticides. Two of the *non-Bt* cotton fields were treated with pesticides (P, N) and the other two did not receive applications (H and USDA). One of the commercially managed pairs of fields (*Bt* and *non-Bt*) received 14 applications of endosulfan for boll weevil control at low rate throughout the season and six early applications of Vydate™ (16 oz/ acre; a.i. oxamyl). A second pair received five applications of Guthion™ (16 oz/acre a.i. methylazinphos) during early and mid-season. The third *Bt* commercial field (AM) was treated with 3 applications of Orthene™ (Valent, a.i. acephate), 2 of Guthion™ and 2 of Baythroid™ (Bayer, a.i. cyfluthrin); the corresponding paired field (USDA) did not receive pesticide applications.

Statistical analysis

Number of larvae (total, live or dead) and damage in Bt vs. non-Bt cotton: Data were analyzed using analysis of covariance. Within each analysis, plant structure (boll, flower, square and terminal) and plant phenotype (*Bt* cotton and *non-Bt* cotton) were defined as class variables with days after planting (plant age) as the covariate. Response variables were the numbers of heliothine larvae (live, total dead, dead neonates plus dead first instars, or dead neonates plus dead first and second instars) or numbers of damaged plant structures. Individual fields represented replicates.

Larval vertical distribution in Bt vs. non-Bt cotton: The data of number of heliothine larvae were analyzed by analysis of covariance with *Bt* cotton versus conventional (non- *Bt)* cotton and node number as independent variables. Because the numbers of nodes per plant varied between plants within sample dates, data were transformed so that all plants contained 20 nodes, which was equivalent to the average number of nodes per plant among samples (mean = 18.7 nodes, SD = 3.5). Total numbers of larvae, damage, total number of live larvae or total number of dead larvae was dependent variables, each examined separately. Days after planting (DAP) was the covariate. The interest in this analysis was not whether there are more larvae in *Bt* than non-Bt cotton and not simply whether there are more total larvae, damage, live or dead larvae in one node position relative to another but whether there is a significant interaction of *Bt* vs. non-Bt with node position (looking at total larvae, live, dead and damage). In other words, do the vertical distributions of total number of larvae, damage, live larvae or dead larvae differ between *Bt* and non-Bt cotton?

Results and discussion

Total number of larvae, dead and live, and plant damage in Bt and non-Bt cotton: In the graphs, the densities of total larvae, dead or live are presented as the number of larvae per 100 plants. Abbreviations in the graphs are defined as N=total number of neonates; N+1= total number of neonates plus first instar larvae; N+1+2= total number of neonates plus first and second instar larvae.

A significantly greater number of larvae (both dead and alive) were found in *Bt* cotton than non-Bt cotton fields, with more larvae per plant in the conventional cotton fields (2.23 ± 0.90 larvae/ 100 plants) vs. 0.48 ± 0.11 in *Bt* fields (Figure 1). There were no detectable differences in the number of total larvae found in diverse plant structures (flower, boll, square or terminal) in *Bt* cotton vs. non-Bt cotton. There was no detectable interaction between treatments and plant structures.

Similar trends were obtained for the total number of live heliothine larvae; significantly more larvae were found in the conventional cotton fields (2.27 ± 0.89 larvae/ 100 plants) compared to numbers found in the *Bt* cotton fields (0.48 ± 0.11) (Figure 2). Keeping with these trends, significantly more dead larvae were found in *Bt* cotton fields (0.22 ± 0.06 larvae/ 100 plants) than in non-Bt fields (0.01 ± 0.01 larvae) (Figure 3). No detectable differences were found between plant structures, and there was no significant interaction of treatment (*Bt* vs. non-Bt) with plant structures.

In examining the numbers of dead neonate and 1st instar larvae (= N+1) by treatment and plant structure, several interesting patterns were detected (Figure 4). First, significantly greater numbers of dead larvae were discovered in *Bt* fields compared to non-Bt fields. The numbers of dead larvae found in terminals was statistically greater than the numbers found in bolls and flowers, but was not different from the numbers in squares. No statistically significant differences were detected among squares, bolls, and flowers for the number of dead neonates and first instar

larvae. Third, there was a significant interaction between treatment and plant structure, indicating that the magnitude of these differences found among plant structures varied between *Bt* and non-Bt fields. Similar results were obtained when 2nd instars (=N+1+2) were included in the analysis (not shown). There were significantly more damaged structures in the non-Bt cotton (6.14 ± 0.18 damaged plants/ 100 plants sampled) than in *Bt* cotton (1.89 ± 0.18) (Figure 5). These differences were consistent among plant structures. No interaction between treatment and plant structures was detectable.

Vertical distribution of larvae and damage in Bt vs. non-Bt cotton: There is not a significant interaction for the number of total larvae (dead + alive) in *Bt* vs. non-Bt cotton (not shown), indicating that the total larval distribution is comparable between *Bt* and non-Bt fields. This may reflect the fact that the oviposition behaviour of female moths is similar for both, *Bt* and non-Bt cottons. There are, however, significant interactions for the number of live and dead larvae and for damaged structures. The vertical distribution of live heliothine larvae was found to be different in *Bt* cotton than in non-Bt cotton. There are significantly more live larvae toward the tops of the non-*Bt* plants than in *Bt* plants. Correspondingly, there is significantly more damage toward the top of the plant in the non-Bt compared with *Bt* cotton. There are significantly many more dead larvae at the tops of *Bt* plants than in non-Bt plants. In *Bt* cotton, the terminal appears to be highly toxic to heliothine larvae. This was later confirmed by toxin content estimations (Greenplate, 1999). In *Bt* cotton, live heliothine larvae are mainly present towards the middle of the plant.

Acknowledgements

Funding by Cotton, Inc., U.S., is greatly appreciated. The assistance of Lori Nemec, Eli Crow and Brian Grote is recognised.

Reference

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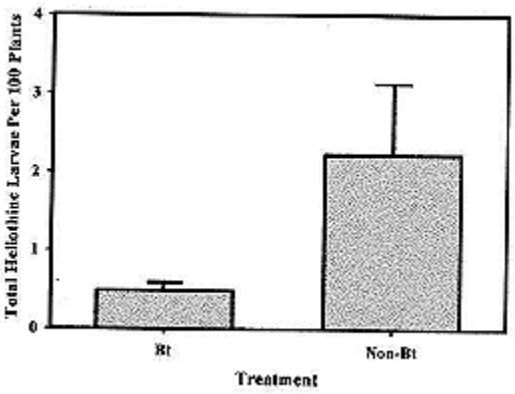
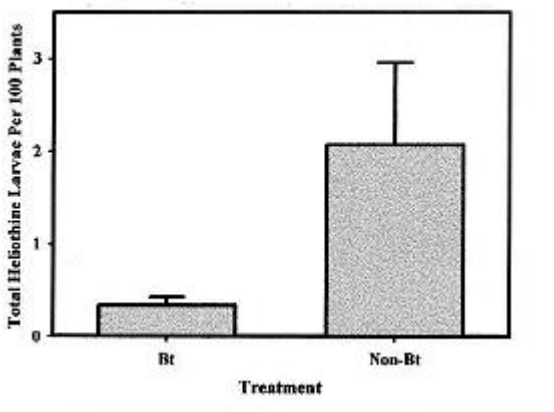
Figure 1. Mean and standard deviation of total heliothine larvae in *Bt* and non-Bt cotton fields.



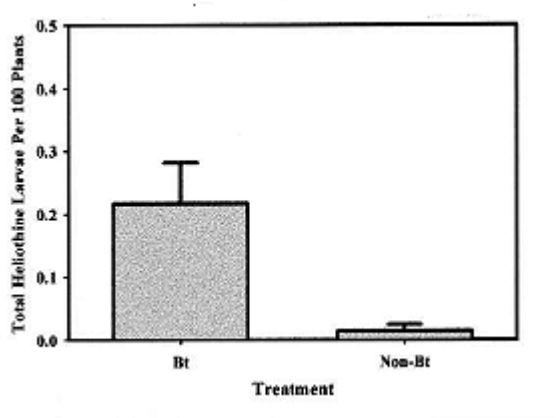
F (Treatment) – 4.41; df – 1,271; p – 0.0367
F (Plant structure) – 1.35; df – 3,271; p – 0.259
F (Treatment x Plant structure) – 0.95; df – 3,271; p – 0.416

Figure 2. Mean and standard deviation of live larvae in Bt and non-Bt cotton.



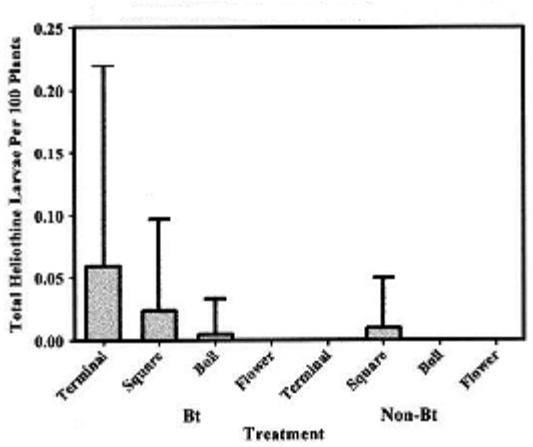
F (Treatment) – 4.41; df – 1,271; p – 0.0367
F (Plant structure) – 1.35; df – 3,271; p – 0.259
F (Treatment x Plant structure) – 0.95; df – 3,271; p – 0.416

Figure 3. Mean and standard deviation of number of dead lepidopteran larvae, all instars.



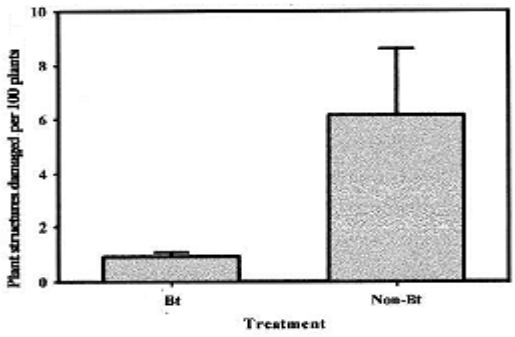
F (Treatment) – 10.19; df – 1,271; p – 0.002
F (Plant structure) – 1.30; df – 3,271; p – 0.277
F (Treatment x Plant structure) – 1.67; df – 3,271; p – 0.173

Figure 4. Mean and standard deviation of dead neonates and first instar larvae by plant structure in Bt and non-Bt cotton.



F (Treatment) – 6.01; df – 1,271; p – 0.015
F (Plant structure) – 3.292; df – 3,271; p – 0.021
F (Treatment x Plant structure) – 3.18; df – 3,271; p – 0.02

Figure 5. Mean and standard deviation of damaged structures per 100 plants in Bt and non-Bt cotton.



F (Treatment) – 5.25; df – 1,271; p – 0.023 F (Plant structure) – 1.47; df – 3,271; p – 0.224 F (Treatment x Plant structure) – 1.21; df – 3,271; p – 0.307