DISTRIBUTION OF HELIOTHINE LARVAE IN *B. t.* AND NON-*B. t.* COTTON IN TEXAS

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Abstract

We conducted a study to assess larval survivorship among diverse cotton plant structures in *B.t.* vs. conventional cotton. This study was motivated by *B.t.* cotton failures in the Brazos River Bottom in 1996, when bollworm *(Helicoverpa zea)* larvae were found in flowers, apparently feeding on pollen with low toxin content. In 1997 and 1998, *B.t.* cotton varieties carrying the Cry1Ac toxin gene and non-*B.t.* cotton were scouted in field pairs in four locations in the Brazos River Bottom around College Station, TX. Larvae were classified as found on terminals, squares, flowers and bolls. The node position was also recorded. Our data indicate that the majority of the larvae are found in terminals and squares in *B. t.* cotton, and that the terminal is a critical factor in the mortality of heliothine larvae exposed to *B. t.* cotton. Third instar larvae found in flowers in *B.t.*cotton were identified either as *H. zea* or *Spodoptera spp.*, as expected. The vertical distribution of live larvae is different in *Bt* cotton than in conventional cotton. In *Bt* cotton live larvae are found towards the middle of the plant, mainly in squares, the most damaged structures.

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 (B. t.)* 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 *B. t.* toxin available in diverse plant tissues. 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. 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. 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 *B. t.* cotton canopy.

Survival of larvae in diverse plant structures may alternatively indicate the presence of Cry1Ac resistant individuals. Laboratory detection of resistant larvae of bollworm (*Helicoverpa zea)* is complicated by the fact that the natural tolerance of this species is highly variable. By contrast, the Cry1Ac toxin exhibits a high potency for tobacco budworm (*Heliothis virescens*). 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. We investigated the spatial distribution of Heliothine larvae in *B.t.* cotton during the second and third year after the introduction of commercial *B. t.* 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 *B.t.* 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 *B .t.* cotton/ refuge plan. 2. Compare the vertical distributions of heliothine larvae in conventional and *B. t.* 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, cotton varieties carrying the Bollgard™ gene (Cry1Ac toxin) and conventional non-*B. t.* cotton were scouted in pairs in four locations in the Brazos River Bottom around College Station, TX. The total effort exerted during the season was 79.5 person x h for conventional cotton and 64.75 person x h for *B. t.* cotton; 2875 plants were scouted in non-*B. t.* cotton and 2825 plants in *B.t.*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 cotton fields were commercially managed pairs and received pesticide applications against lepidopterans. Pairs (*B. t* and non-*B. t.)* of fields received various (maximum of 14) applications of endosulfan for boll weevil control at low rate throughout the season, and various (up to 6) early applications of Vydate™ (16 oz/ acre; a.i. oxamyl), and various (up to 5) applications of Guthion™ (16 oz/acre; a. i. methylazynphos) during early and mid-season. In 1998, 6 pairs of *B. t.* and *non-B. t.* cotton fields were scouted in the same area following the same methodology. Each field was scouted once a week and throughout the season; 1,200 plants were scouted in *B.t.*and non-*B.t.* cotton fields, respectively. Cultivars were

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non-*B.t.* Delta Pine 5415 (parent) and *B. t.* Delta Pine NuCOTN 33^{B}, seeded at 40" between rows. The six pairs of fields were managed using conventional pest control practices, with various degrees of insecticide application as needed. Pairs of *B.t.* and non-*B.t.* cotton plots commercially managed received aerial applications of Vydate™ (16 oz/ acre; a.i. oxamyl, systemic), Guthion™ (16 oz/acre; a. i. methylazynphos), and Furadan™ (6 oz/ acre; a. i. carbofuran) or Provado™ (Bayer, 3¾ oz/ acre; a. i. imidacloprid) against aphids. Fields were irrigated 2-4 times during the season. Fields typically received four applications of endosulfan for boll weevil control during May and June.

Statistical Analysis

Number of Larvae (Total, Live or Dead) and Damage in B.t. Vs. Non-B.t. Cotton: data were analyzed using analysis of covariance. Within each analysis, plant structure (boll, flower, square, and terminal) and plant phenotype (*B.t.* cotton and non-*B.t.* 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 B.t.vs. Non-B.t. Cotton: The data of number of heliothine larvae were analyzed by analysis of covariance with *B. t.*-cotton versus conventional (non-*B.t.)* 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 number of larvae, damage, total number of alive larvae or total number of dead larvae were dependent variables, each examined separately. Days after planting (DAP) was the covariate. In this new analysis we are not looking at whether there are more larvae in *B. t.* vs. non-*B. t.* cotton, and we are not simply interested in whether there are more total larvae, damage, live or dead larvae in one node position relative to another. What we are interested in is determining whether there is a significant interaction of *B.t.* vs. non-*B.t.* 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 *B. t.* vs. non-*B. t.* cotton?.

Results and Discussion

1997 Total Number of Larvae, Dead and Live, and Plant Damage in *B. t.* and Non *B.t.* Cotton

In the graphs below, the densities of total number of larvae, dead or live are presented as the number of larvae per 100 plants. Abbreviations used 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. As shown in Fig. 1, a significantly greater number of larvae (both dead and alive) were found in *B. t.* cotton vs. non *B. t.* 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 *B. t.* fields (Fig. 2). There were no detectable differences in the number of total larvae found in diverse plant structures (flower, boll, square or terminal) in *B. t.* cotton vs. non *B. t.* 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 *B. t.* cotton fields (0.48 ± 0.11). Keeping with the above trends, significantly more dead larvae were found in *B. t.* cotton fields (0.22 ± 0.06 larvae/ 100 plants) than in non-*B. t.* fields (0.01 ± 0.01 larvae) (Fig. 3). No detectable differences were found between plant structures, and there was no significant interaction of treatment (*B.t.* vs. non *B.t.*) 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 (Fig. 4). First, significantly greater numbers of dead larvae were discovered in *B. t.* fields compared to non-*B. t.* fields. Second, the numbers of dead larvae found in terminals was statistically greater than the numbers found in bolls and flowers, but no 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 *B. t.* and non-*B. t.* 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-*B. t.* cotton (6.14 ± 0.18 damaged structures/ 100 plants sampled) than in *B. t.* cotton (1.89 ± 0.18) (Fig. 5). These differences were consistent among plant structures. No interaction between treatment and plant structures was detectable in 1997 for the number of damaged structures.

1998 Total Number of Live Larvae and Plant Damage in *B. t.* and Non *B. t.* Cotton

As in 1997, there were significant differences in the number of live larvae between *Bt* and conventional cotton in 1998 (Fig. 6). However, in 1998 there were significant differences in the number of live larvae among structures in *Bt* vs. conventional cotton, and there was a significant interaction between treatments (*B.t.* vs. conventional cotton) and plant structures. Accordingly, the results of damaged structures reflect closely the results obtained with live larvae (Fig. 7), indicating that the level of damage to diverse structures is different in *B.t.* vs conventional cotton. Note that the number of larvae found in flowers in *Bt* cotton is minimal (Fig. 7).

1997 Vertical Distribution of Larvae and Damage in *B. t.* vs. Non *B. t.* Cotton

The following graphs reflect the average distribution of larvae (total, dead and alive) and damage for plants where these variables were different than zero. There is not a significant interaction for the number of total larvae (dead + alive) in *B. t.* vs. non *B. t.* cotton, indicating that the total larval distribution is comparable between *B. t.* and non *B. t.* fields (Fig. 8). This may reflect the fact that the oviposition behavior of female moths is similar for both, *B.t.* and non-*B.t.* cottons. There are, however, significant interactions for the number of live and dead larvae, and for damaged structures (Figs. 9-11). The vertical distribution of live heliothine larvae was found to be different in *B. t.* cotton than in non *B. t.* cotton (Fig. 9). There are significantly more live larvae toward the tops of the non-*B. t.* plants than in *B. t.* plants. Correspondingly, there is significantly more damage toward the top of the plant in the non-*B. t.* cotton compared to *B. t.* cotton (Fig. 10). There are significantly many more dead larvae at the tops of *B. t.* plants than in non *B. t.* plants (Fig. 11). In *B. t.* cotton, the terminal appears to be highly toxic to heliothine larvae. In *B. t.* cotton, alive larvae of heliothines are mainly present towards the middle of the plant and mainly in squares, the most damaged structures in 1998.

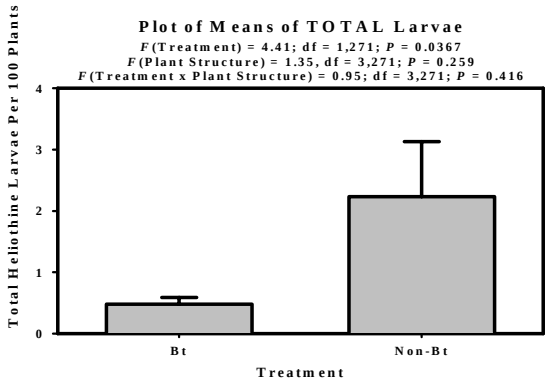

Figure 1. Total number of heliothine larvae in *B. t.* and non-*B. t.* cotton fields in 1997.

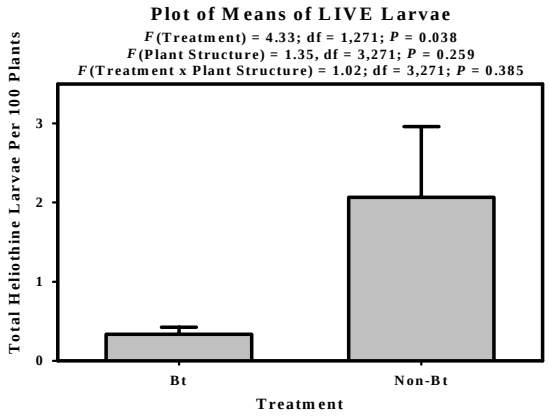

Figure 2. Total number of live larvae in *B. t.* and non *B. t.* cotton in 1997.

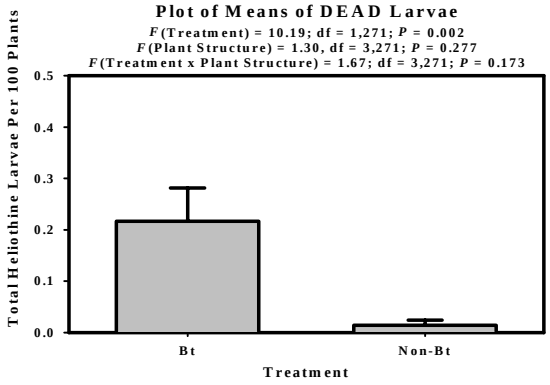

Figure 3. Total number of dead lepidopteran larvae, all instars in 1997.

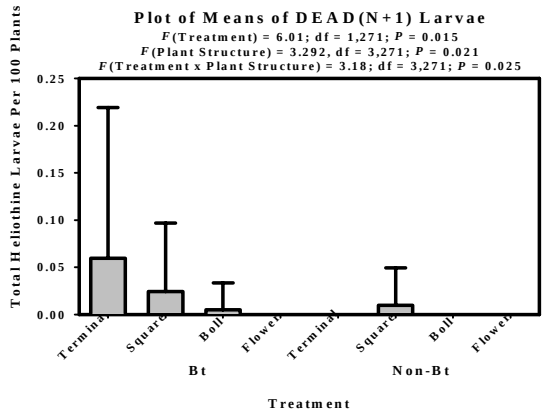

Figure 4. Total number of dead neonates and first instar larvae by plant structure in *B.t.*and non *B. t.* cotton 1997.

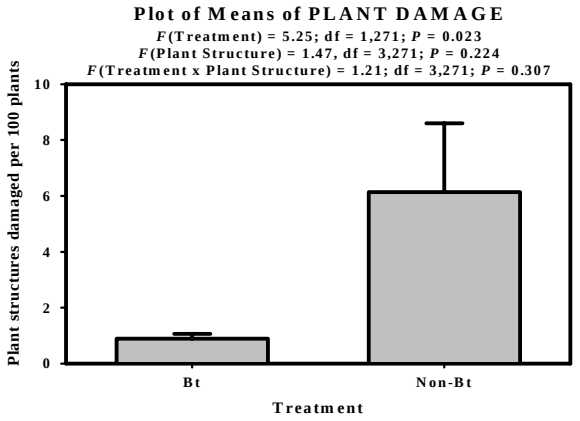

Figure 5. Number of damaged structures per 100 plants in *B. t.* and non *B. t.* cotton in 1997.

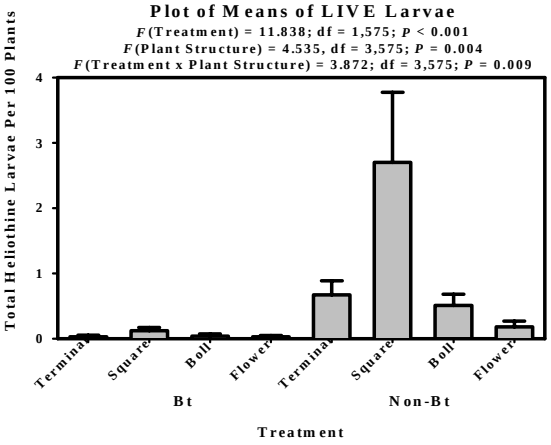

Figure 6. Number of live larvae in diverse structures in 1998.

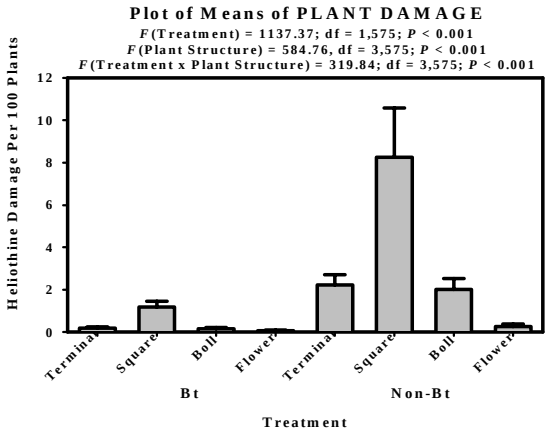

Figure 7. Number of damaged structures per 100 plants in 1998.

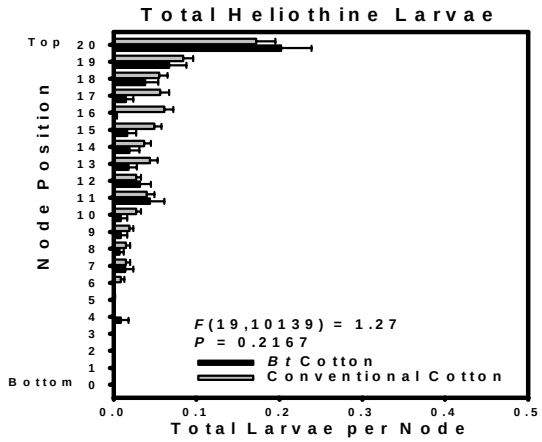

Figure 8. Vertical distribution on Total heliothine larvae in 1997.

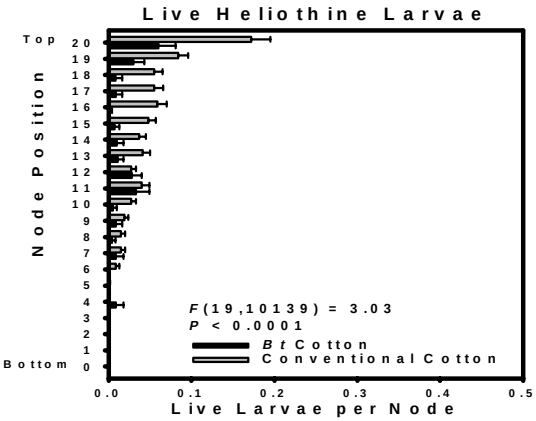

Figure 9. Vertical distribution of Live heliothine larvae in 1997.

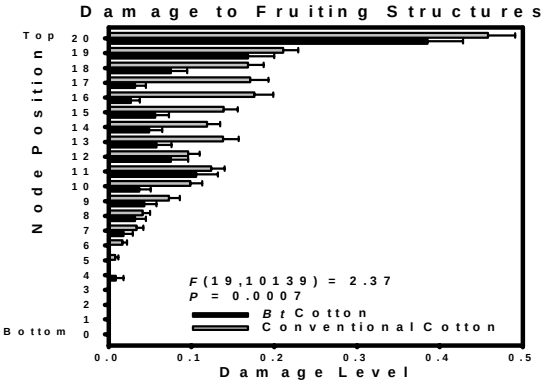

Figure 10. Vertical distribution of number of structures damaged per 100 plants in 1997.

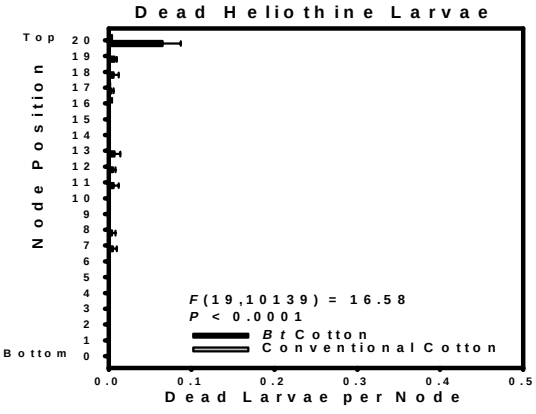

Figure 11. Vertical distribution of numbers of dead larvae in 1997.

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