BOLLWORM (*HELICOVERPA ZEA*) DAMAGE AND BEHAVIOR IN BT COTTON

R. Godbold
J. Gore
D. Cook
Mississippi State University
Delta Research and Extension Center
Stoneville, MS
F. Musser
A. Catchot
Mississippi State University
Dept. of Biochemistry, Molecular Biology, Entomology, and Plant Pathology
Starkville, MS
D. Dodds
Mississippi State University
Dept. of Plant and Soil Science
Starkville, MS

Abstract

Use of Bt technology has played a major role in the control of bollworm. Variation in expression levels along with selection pressure on field populations has led to the development of large-scale resistance to Bt technology. Trials were conducted to evaluate how Bt genes affect bollworm larvae in flowering cotton. Based on the lack of sensitivity and decrease in selective feeding of bollworm, the use of Cry1Ac and Cry2Ab in cotton varieties shows some similarities in feeding levels when compared to non-Bt cotton. Addition of Vip3A into the plant technologies has the greatest effect for control of larvae.

Introduction

The use of genetically engineered crops expressing insecticidal properties play a major role in agronomic production systems by contributing to integrated pest management. *Bacillus thuringiensis* var. *kurstaki* (Bt) is currently being used in cotton for its insecticidal effects on various pests. *B. thuringiensis*, a natural occurring soil-dwelling bacterium, has numerous proteins that provide control of different pests or combinations of pests. Bollgard® (Monsanto Co., St. Louis, MO) cotton was the first cotton cultivar that incorporated Bt toxins within the crop genetics. A single protein (Cry1Ac) was used (Perlak et al. 1990). The use of Cry1Ac provided control of tobacco budworm, pink bollworm, and bollworm (MacIntosh et al. 1990). Upon the commercial availability of Bollgard cotton, researchers and agricultural pest managers noted that *H. zea* larvae responded differently to cotton expressing Bt proteins than conventional cotton (Gore et al. 2002). Larvae moved to areas with low Bt expression; increasing their chances of survival and development. Survival of bollworm populations on Bollgard® cotton led to the increase in Bt resistance. Additional Bt toxins were added to improve resistance management and increase control of bollworm populations. The Cry2Ab protein from *B. thuringiensis* was added to the original Cry1Ac protein in Bollgard cotton to make Bollgard II cotton. Bollgard II cotton has provided improved control of bollworm and other lepidopteran pests (Stewart and Knighten 2000; Gore et al. 2001). The development of Bt resistance to Bollgard II cotton has increased in recent years (Reisig et al. 2018). Similar to Bollgard cotton, expression levels of Cry1Ac and Cry2Ab decline over time in Bollgard II (Carrière et al. 2019). Cotton cultivars expressing a third Bt toxin have now been incorporated into the agricultural production system. The addition of the *B. thuringiensis* vegetative insecticidal protein, Vip3A, to current cotton cultivars targets a different binding site within the midgut of *H. zea* and provides better control than dual-gene cultivars (Lee et al. 2003). Vip3A provides a novel site of action to combat Bt resistant bollworm populations.

Materials and Methods

Three cultivars of cotton were planted in strip plots representing non-Bt (Deltapine 1822XF, Bayer CropScience, St. Louis, MO), Bollgard II (Deltapine 1646B2XF), and Bollgard 3 (Deltapine 1835B3XF). Infestations and observations were conducted during the flowering stages of the different planting dates. Individual plants were selected based on the presence of a first position white flower. Plants selected for infestation were isolated from other plants by cutting adjacent plants at the soil surface and removing them from the plot area. Nodes containing the first position white flower were marked with yellow slant and lock labels (#HHC31YE, A. M. Leonard, Inc., Piqua, OH) and numbered prior to infestation. Isolated plants were then supported with metal home garden stakes pushed into the ground at the

base of the plant and zip tied to the plant on one of the upper nodes for support. Prior to infestation, approximately 30 newly eclosed *H. zea* larvae were placed in large plastic cups containing a thin layer of diet 24 ± 6 h prior to being placed in white flowers. For infestations, first-instar *H. zea* larvae were individually placed into the previously marked white flowers with a fine bristle artists paint brush. Larvae were allowed to feed before infestation to minimize mortality from handling in the field. Larvae from the first, second, and third laboratory generations were used throughout the experiment. Second laboratory generation larvae were used for third and fourth replications. Finally, third laboratory generation larvae were used for the fifth and sixth replications. All replications were conducted on different days. Observations were made at three, seven, nine, and eleven days after infestations. Observations consisted number of damaged fruiting structures based on each individual larva. Damaged sites were noted to keep track of amounts of damage being conducted and to help track larvae moving throughout the plant. Additional observations were made during the second year to evaluate bloom tag losses at the three-day observation time. All data were analyzed with a generalized linear mixed model (Proc Glimmix, SAS ver. 9.4; SAS Institute; Cary, NC). Degrees of freedom were calculated using the Kenward-Rogers method.

Results and Discussion

Larval Recovery

Larval recovery was affected by the interaction between cultivar and observation date ($P < 0.01$). Initial recovery (3 d) of larvae was different among all cultivars with 62, 38, and 15% of larvae being recovered on non-Bt, Bollgard II, and Bollgard 3 cotton, respectively (Fig. 1). In general, percent recovery declined gradually over time for non-Bt and Bollgard II cotton. For Bollgard 3 cotton, percent recovery declined from 3 to 7 d, but recovery at 9 and 11 d was similar to that observed at 7 d. At 7 d after infestation, differences were still observed among all cultivars. At 9 and 11 d after infestation, no differences were observed between non-Bt and Bollgard II cotton for percent recovery. Additionally, percent recovery was not different between Bollgard II and Bollgard 3 cotton at those evaluation times. Multiple factors may have contributed to the decline of larval retention. The low level of recovery at 3 d, was likely a result of the loss of infested bloom tags (Fig. 2). Percentages of shed bloom tags ($P < 0.01$) differed significantly between varieties. Reasons for these occurrences are unknown. However potential causes may include varietal factors or indirect results caused by larval feeding in bloom tags. General movement behavior along with plant isolation may have played a key factor in losses of larvae. Under normal field conditions, larval recovery may increase due to more surface area provided by cotton plants overlapping in any given area.

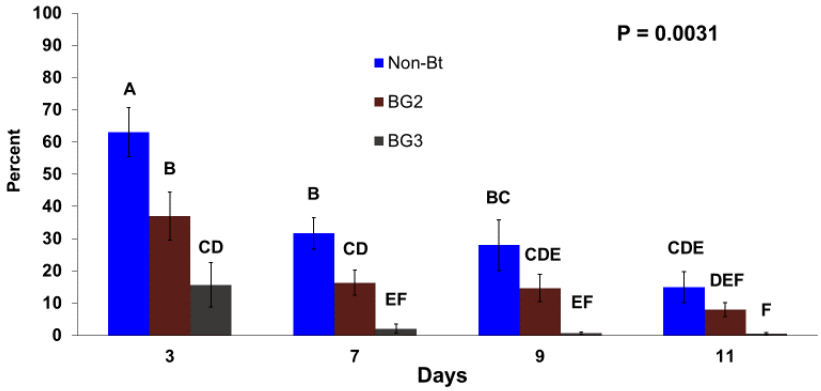

Figure 1. Larval recovery over time in non-Bt, Bollgard II, and Bollgard 3 cottons

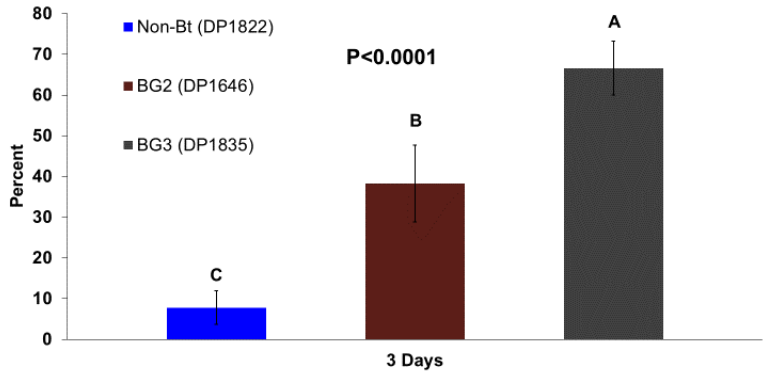

Figure 2. Bloom tag losses after three days

Damage

Cumulative fruiting form damage was recorded throughout the experiment. A cultivar by observation date interaction ($P < 0.01$) was observed for fruiting form damage. At the initial observation timing (3 d), no differences were observed among cotton cultivars for fruiting form damage (Fig. 3). At 7 and 9 d, no differences in fruiting form damage were observed between non-Bt cotton and Bollgard II cotton. At 7 d, damage was not different between Bollgard II and Bollgard 3, but Bollgard 3 had less damage than Bollgard II at 9 d. At eleven days, Bollgard II had less overall damage than non-Bt cotton and Bollgard 3 cotton had less damage than both non-Bt and Bollgard II cottons. At the 11 d evaluation timing, total damage in non-Bt cotton averaged 4.6 fruiting forms. For Bollgard II, total damage averaged 3.4 fruiting forms. Bollgard 3 had the least amount of damage with an average of 1.6 fruiting forms Although larval size was not quantified to minimize disturbance, larvae on Bollgard II cotton appeared to be smaller in size compared to larvae on non-Bt cotton. Larvae on Bollgard 3 cotton appeared to be the smallest. This may be the result of Bt toxins affecting the rate of larval growth and development as seen in other studies

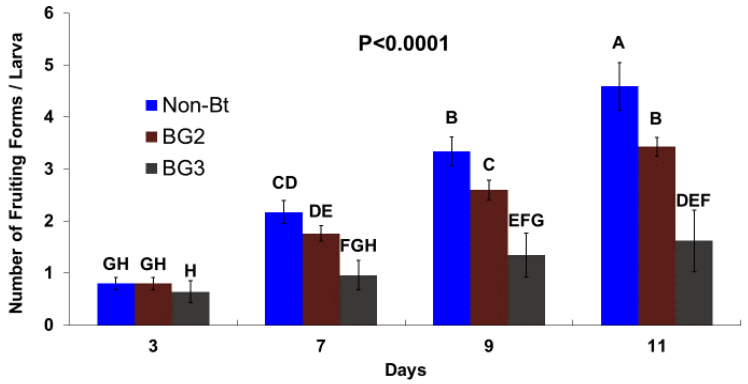

Figure 3. Total fruiting form damage per larva over time

No differences in total boll damage were observed between Bollgard II and non-Bt (Fig. 4). Bollgard II had an average of 2.6 bolls damaged per larva, whereas non-Bt had 2.9 damaged per larva. Bollgard II did provide reductions in square damage. This is likely the result of higher expression of Bt in young fruiting forms (Greenplate 1999, Adamczyk et al. 2001, Adamczyk and Sumerford 2001). Control of bollworm has declined in Bollgard II and other two-gene cottons. Damage has increased for all fruiting forms when compared to the performance of Bollgard II

during the year 2000 (Fig. 5). Bollgard 3 did showed decreases in damage conducted by individual larvae for both squares and bolls and proved to be most effective.

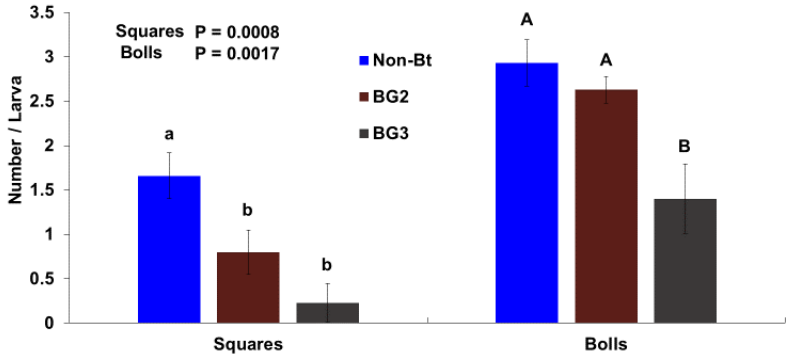

Figure 5. Damage separated by fruiting form type

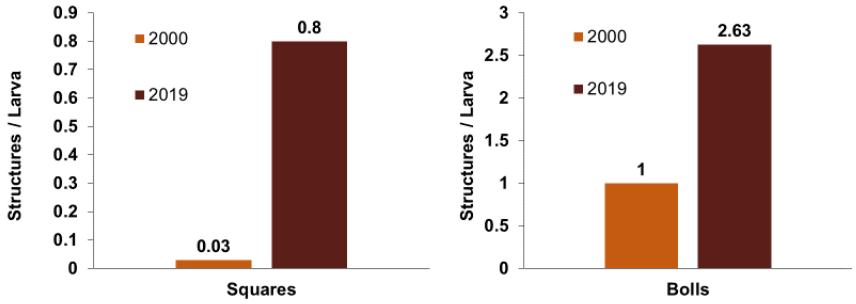

Figure 4. Bollgard II damage per larva comparison between the years 2000 and 2019

Summary

Our results concluded that Bollgard II damage has increased since the study conducted by Gore et al. (2003). The findings of this experiments support the need for supplemental control in Bollgard II cotton (Reisig et al. 2018) to prevent damage as seen by Kerns et al. (2018). Bollgard 3 will provide better control and damage prevention under high pressure from bollworm populations. However, it is possible that the effectiveness of Vip 3A cotton could eventually be compromised due to larval behavior that was seen after previous Bt crop technologies were introduced and resistance to Cry toxins (Reisig et al. 2018).

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