

## ARTHROPOD MANAGEMENT

### Impact of Bollworms [*Helicoverpa zea* (Boddie)] on Maturity and Yield of Bollgard Cotton

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#### ABSTRACT

**A large percentage of Bollgard cotton is treated for bollworms [*Helicoverpa zea* (Boddie)] with little information about economic losses from these infestations. The impact of bollworm infestations on maturity and yield of Bollgard cotton was determined. Infestations of 1-d-old bollworm larvae were established on non-Bollgard and Bollgard cottons in large field cages. Treatments included three and five levels of infestation for 1 to 4 wk in 2002 and 2003, respectively. Bollworms significantly delayed maturity of Bollgard cotton when 100% of white flowers were infested for 1 to 4 wk or when 50% of white flowers were infested for 2 to 4 wk in 2002. When averaged across weeks, bollworms delayed maturity of Bollgard cotton when 100% of white flowers were infested in 2003. Yield responses of Bollgard cotton varied between years, but yields tended to decline as the level of infestation increased each year. There was a significant negative relationship between the cumulative numbers of white flowers infested and seedcotton yields in Bollgard cottons. The resulting regression equation from this relationship had a slope of -1.69, indicating a 1.69 g reduction in yield for every white flower infested. Results of this study will be important for refining action thresholds for bollworms on Bollgard cotton and suggested controls should be initiated before infestation levels reach greater than 25% of white flowers. These data also provide a base of information for predicting yield reductions caused by bollworm infestations in white flowers of Bollgard cotton.**

**T**he bollworm [*Helicoverpa zea* (Boddie)] and tobacco budworm [*Heliothis virescens* (F.)] are key pests of cotton throughout much of the

southeastern and mid-southern United States. Historically, insecticides have been the primary tool used to manage these insects in cotton, but the widespread occurrence of resistance to organophosphates and pyrethroids during the early 1990s in the tobacco budworm made management difficult (Leonard et al., 1988; Plapp et al., 1990; Elzen et al., 1992). During the 1996 growing season, genetically engineered cottons that revolutionized integrated pest management in cotton were introduced for commercial production (Perlak et al., 2001). These novel cottons produce the Cry1Ac protein from the soil bacterium [*Bacillus thuringiensis* Berliner var. *kurstaki*] and are sold under the trade name Bollgard (Monsanto Co.; St Louis, MO.) (Perlak et al., 1990). The Cry1Ac protein provides good control of the tobacco budworm, and insecticide applications have not been needed to manage this pest on Bollgard cotton. In contrast, insecticide applications often are needed in Bollgard cotton to prevent economic losses from bollworms.

Several factors contribute to the pest status of bollworms on Bollgard cotton. First, bollworms are less susceptible to the *B. thuringiensis* Cry1Ac protein than tobacco budworms (Luttrell et al., 1999). Also, temporal and spatial variations in the expression of Cry1Ac among different plant parts results in some structures having lower expression than other structures (Greenplate, 1999; Adamczyk et al., 2001). Populations of bollworms surviving in Bollgard cotton tend to be associated with white flowers and small bolls within 1 to 2 d after anthesis (Gore et al., 2000). Previous research has shown that bollworm larvae move among different structures more frequently in Bollgard cotton than non-Bollgard cotton (Gore et al., 2002) and feed on structures (white flowers and small bolls) where their chance of survival is greatest (Gore et al., 2001). As a result, agricultural consultants and pest management specialists have adjusted their scouting protocols for Bollgard cotton. In non-Bollgard cotton, insecticide applications generally are based on damaged squares and numbers of live larvae found in terminals and squares, and insecticide applications in Bollgard cotton are based on numbers of live larvae in

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small bolls. Also, some states recommend treatment based on numbers of eggs or numbers of white flowers with bollworms. This manuscript reports field-cage experiments designed to determine the impact of bollworm level and duration of infestation on maturity and yield of Bollgard cotton.

## MATERIALS AND METHODS

Three separate experiments were conducted with non-Bollgard and Bollgard cotton in 2002 and 2003. During 2002, separate experiments were conducted with non-Bollgard (Stoneville 4793 RR; Stoneville Pedigreed Seed; Memphis, TN) and Bollgard (Stoneville 4892 BR) cottons planted into separate large (0.05 hectare) field cages on 14 May 2002. During 2003, Bollgard cotton (Suregrow 215 BR; Delta Pine and Land Co.; Scott, MS) was planted into a large field cage on 8 May 2003. Non-Bollgard cotton was included in 2002 to validate the procedure of artificially infesting larvae into white flowers. First instar bollworms are not typically observed in white flowers of non-Bollgard cotton (Gore et al., 2002); therefore, non-Bollgard cotton was not included in 2003. Plot size was two rows (101.6-cm centers) by 1 m with a 2-m alley between plots. Also, plots were separated by one non-planted row to minimize inter-plot migration of larvae. Plant densities were thinned to 12 plants per plot (6 plants per m row) 2 wk after plant emergence.

The experimental design was a randomized complete block with three replications. Each cage was separated into three sections. Each experiment was arranged so that all treatments within a replication were planted in the same section. Plots were planted in a split-plot arrangement. Duration of infestation (weeks) was the main-plot factor and included weekly intervals for 1 to 4 wk during the flowering period. Cotton growth stages ranged from nine to five nodes above white flower during the 4-wk period in 2002, and nine to four nodes above white flower during the 4-wk period in 2003. Nodes above white flower counts were determined by counting the number of main stem nodes above the upper-most first position white flower as described by Bourland et al. (1992). Level of infestation was the sub-plot factor. The white flower infestation levels included 0, 50, and 100% during 2002. During 2003, the study was expanded to include infestation levels of 0, 10, 25, 50, and 100%. Crop development was monitored throughout the season to determine the initiation of

flowering and the proper time for artificial infestation of larvae. The entire test area was treated with insecticides weekly until 2 wk prior to artificial infestations to minimize injury from natural infestations of insect pests and eliminate natural enemies. Two weeks before artificial infestations, the cages were covered with translucent 32 mesh Lumite screen (Synthetic Industries; Greenville, GA).

To obtain sufficient numbers of larvae at the proper stage for infestation, a colony of bollworms from field corn (*Zea mays* L.) was established each year. Approximately 200 to 300 large ( $\geq 4^{\text{th}}$  instar) larvae were collected from corn ears each day for 5 d. Larvae were transported to the laboratory and maintained for one generation before plots were artificially infested. Larvae were fed a wheat germ-based meridic diet (King and Hartley, 1985) and maintained at  $27 \pm 2^{\circ}$  C,  $80 \pm 5\%$  relative humidity, and a photoperiod of 14:10 (L:D) h. After larvae completed development, pupae were put into 3.8-L cardboard containers (about 50 per container). The tops of the containers were covered with batiste cloth (egg sheet) to serve as an oviposition substrate. The egg sheets were harvested daily and placed into 3.8-L plastic bags. Upon eclosion, neonates were offered meridic diet in 236-ml cardboard cups (about 100 per cup). Larvae were allowed to feed for  $24 \pm 4$  h before field use to minimize mortality from handling neonates in the field.

Infestations of larvae were initiated when plots across the test area averaged nine nodes above white flower. This corresponded to 24 and 14 July in 2002 and 2003, respectively. The total numbers of white flowers were counted in each plot and larvae were placed into white flowers (one larva per flower) with a paint brush corresponding to the level of infestation for each plot. Larvae were placed into white flowers daily for the designated number of weeks and allowed to feed unhindered. If a plot did not have enough flowers on a particular day to achieve the desired level of infestation, a running total of flowers was maintained for each plot to obtain the desired level of infestation on subsequent days. At the end of the season, the percentage of open bolls was determined as a measure of crop maturity by counting the total number of open and closed bolls in each plot. The percentage of open bolls was determined for each plot when the non-infested plots averaged approximately 80% open bolls. Additionally, the plots were harvested by hand and seedcotton weights were determined.

Data from each experiment for percentage open bolls and seedcotton yield were analyzed separately with analysis of variance (PROC MIXED, version 8.2, SAS Institute; Cary, NC). In the model, duration of infestation (main-plot), level of infestation (sub-plot), and the duration by level interaction were designated as the fixed components of the model. Replication was designated as a random effect. The replication by duration of infestation interaction was also designated as random and was the error term for duration of infestation. Residual error (replication by duration by level interaction) was the error term for sub-plots and the duration by level interaction. Means were separated using the LSMEANS statement and adjusted according to the Tukey's studentized range test.

In addition to analysis of variance, the relationships between the cumulative numbers of white flowers infested per plot and yield of Bollgard cotton were analyzed with regression analysis (PROC REG, version 8.2, SAS Institute; Cary, NC) in 2002 and 2003. Intercepts and slopes of respective regressions for 2002 and 2003 were compared by analysis of covariance. Consequently, separate regressions with a common slope but different intercepts were calculated for each year. These regressions were calculated to provide an equation for predicting yield losses in Bollgard cotton associated with different levels of white flower infestation.

## RESULTS

**Non-bollgard cotton (2002).** As anticipated, bollworm infestations delayed maturity and reduced yields of non-Bollgard cotton compared with the non-infested plots during 2002. For crop maturity, the level of infestation was significant ( $F = 59.39$ ;  $df = 2, 16$ ;  $P < 0.01$ ) (Table 1). The duration of infestation ( $F = 2.85$ ;  $df = 3, 6$ ;  $P = 0.13$ ) or the duration by level interaction ( $F = 2.34$ ;  $df = 6, 16$ ;  $P = 0.08$ ) was not significant for maturity. When averaged across durations of infestation, the percentage of open bolls was lower when 50 or 100% of white flowers were infested with bollworms compared with the non-infested plots. In addition, the percentage of open bolls was lower when 100% of white flowers were infested than when 50% of white flowers were infested.

Bollworm infestations resulted in yield reductions of non-Bollgard cotton compared with the non-infested plots during 2002. The duration of infestation ( $F = 5.20$ ;  $df = 3, 6$ ;  $P = 0.04$ ) and level of infestation ( $F = 90.65$ ;  $df = 2, 16$ ;  $P < 0.01$ ) were significant (Table 2). The duration by level interaction was significant ( $F = 5.33$ ;  $df = 6, 16$ ;  $P < 0.01$ ), indicating the influence of duration of infestation varied among levels of infestation. Because of this interaction, mean comparisons were made among levels of infestation within each of the durations and among durations within each infestation level. Yields were reduced when 100% of white flowers

**Table 1. Impact of bollworms on maturity of non-Bollgard cotton 2002**

Level of infestation	Mean percentage open bolls ( $\pm$ SEM) <sup>z</sup>				
	1 wk	2 wk	3 wk	4 wk	Mean
0	88.7 (3.3)	89.0 (1.5)	80.7 (4.4)	87.0 (3.8)	86.3 (1.8) a
50	85.3 (3.0)	61.7 (11.7)	55.3 (4.1)	65.3 (9.9)	66.9 (4.8) b
100	70.3 (5.2)	53.7 (6.9)	45.0 (9.1)	44.3 (9.1)	53.3 (4.6) c

<sup>z</sup> Means within a column followed by the same letter are not significantly different according to the Tukey's studentized range test ( $P = 0.05$ ).

**Table 2. Impact of bollworms on seedcotton yield of non-Bollgard cotton in 2002**

Level of infestation	Mean ( $\pm$ SEM) seedcotton yield (g/plot) <sup>z</sup>				
	1 wk	2 wk	3 wk	4 wk	
0	945.5 (23.4) Aa	1052.3 (76.8) Aa	926.8 (47.5) Aa	836.0 (47.7) Aa	
50	957.5 (93.4) Aa	643.2 (118.8) Bb	570.8 (32.9) Bb	714.1 (105.4) ABa	
100	711.1 (36.8) Ab	513.9 (32.6) ABb	399.4 (83.2) Bb	431.4 (50.4) Bb	

<sup>z</sup> Means within a column followed by the same lower-case letter or within a row followed by the same upper-case letter are not significantly different according to the Tukey's studentized range test ( $P = 0.05$ ).

were infested for 1 to 4 wk or when 50% of white flowers were infested for 2 or 3 wk. Reasons for lack of statistical significance at the 50% infestation level for 4 wk are unknown; however, mean yield was approximately 120 g less than the non-infested treatment.

**Bollgard cotton (2002).** Bollworms delayed maturity and reduced yields of Bollgard cotton in 2002. The duration of infestation ( $F = 18.98$ ;  $df = 3, 18$ ;  $P < 0.01$ ) and level of infestation ( $F = 105.17$ ;  $df = 2, 16$ ;  $P < 0.01$ ) were significant for maturity in 2002 (Table 3). In addition, the duration by level interaction was significant ( $F = 4.18$ ;  $df = 6, 16$ ;  $P = 0.01$ ), which indicated a different response to duration of infestation among different levels of infestation. The percentage of open bolls was similar for the non-infested plots among the four durations of infestation. Bollworms reduced the percentage of open bolls when 50 or 100% of white flowers were infested for 2 to 4 wk compared with when 50 or 100% of white flowers, respectively, were infested for 1 wk. In addition, the percentage of open bolls was lower when 100% of white flowers were infested for 3 wk compared with when 100% of white flowers were infested for 2 wk. The percentage of open bolls was lower when 100% of white flowers were infested for 1 to 4 wk compared with the non-infested plots for each of those weeks. In addition, the percentage of open bolls was significantly lower when 50% of white flowers were infested for 2 to 4 wk compared with the non-infested plots within each of those weeks.

For yields of Bollgard cotton in 2002, there were significant main effects for duration of infestation ( $F = 7.42$ ;  $df = 3, 22$ ;  $P < 0.01$ ) and level of infestation ( $F = 80.57$ ;  $df = 2, 22$ ;  $P < 0.01$ ) (Table 4). The duration by level interaction was not significant ( $F = 1.60$ ;  $df = 6, 22$ ;  $P = 0.19$ ). Yields of Bollgard cotton were significantly lower when plots were infested for 4 wk than when plots were infested for 1 or 2 wk. Infestation levels of 50 and 100% of white flowers significantly reduced yields compared with the non-infested plots regardless of duration. In addition, when 100% of white flowers were infested with bollworms, yields were lower than when 50% of white flowers were infested.

**Bollgard cotton (2003).** The impacts of bollworms on maturity or yield of Bollgard cotton in 2003 were not as great as those observed in 2002. For maturity, there was a significant main effect for level of infestation ( $F = 4.37$ ;  $df = 4, 31.2$ ;  $P = 0.01$ ), but not for duration of infestation ( $F = 0.99$ ;  $df = 3, 8.1$ ;  $P = 0.45$ ) (Table 3). Also, the duration by level interaction was not significant ( $F = 12$ ;  $df = 12, 31.2$ ;  $P = 0.35$ ). When averaged across durations, the percentage of open bolls was lower when 100% of white flowers were infested than when 0 or 10% of white flowers were infested.

For yield of Bollgard cotton in 2003, there were significant main effects for duration of infestation ( $F = 11.04$ ;  $df = 3, 39$ ;  $P < 0.01$ ) and level of infestation ( $F = 13.15$ ;  $df = 4, 39$ ;  $P < 0.01$ ) (Table 4). The duration by level interaction was not significant ( $F = 1.49$ ;  $df = 12, 39$ ;  $P = 0.17$ ). Yields were significantly

Table 3. Impact of bollworms on maturity of Bollgard cotton

Level of infestation	Mean percentage open bolls ( $\pm$ SEM) <sup>z</sup>				
	1 wk	2 wk	3 wk	4 wk	Mean
<b>2002</b>					
0	93.0 (1.5) Aa	94.7 (0.3) Aa	88.7 (1.7) Aa	88.7 (2.7) Aa	
50	90.7 (3.2) Aa	77.7 (1.2) Bb	75.0 (1.5) Bb	71.7 (3.5) Bb	
100	80.0 (0.6) Ab	70.7 (3.8) Bb	58.0 (0.6) Cc	65.0 (3.5) BCb	
<b>2003</b>					
0	83.7 (3.5)	78.3 (5.1)	76.7 (1.8)	77.7 (1.8)	79.1 (1.7) a
10	82.7 (2.6)	81.1 (2.3)	79.6 (3.3)	78.3 (2.7)	80.4 (1.3) a
25	79.6 (1.1)	78.1 (3.3)	79.5 (3.9)	74.3 (1.0)	77.9 (1.3) ab
50	78.2 (0.9)	77.1 (1.7)	74.4 (8.4)	74.2 (2.9)	76.0 (2.2) ab
100	77.4 (3.4)	66.3 (6.6)	79.8 (2.3)	67.5 (2.4)	72.7 (2.5) b

<sup>z</sup> Means within a column followed by the same lower-case letter or within a row followed by the same upper-case letter are not significantly different according to the Tukey's studentized range test ( $P = 0.05$ ).

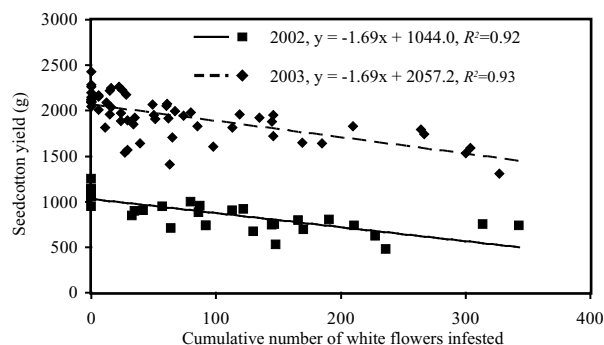
**Table 4. Impact of bollworms on seedcotton yield of Bollgard cotton**

Level of infestation	Mean ( $\pm$ SEM) seedcotton yield (g/plot) <sup>z</sup>				
	1 wk	2 wk	3 wk	4 wk	Mean
<b>2002</b>					
0	1100.6 (17.3)	1166.0 (43.7)	1085.0 (15.5)	1044.5 (55.1)	1099.1 (20.6) a
50	881.9 (18.4)	885.6 (89.2)	852.6 (58.7)	721.9 (25.2)	835.6 (31.1) b
100	856.4 (61.4)	764.8 (36.5)	631.2 (61.1)	655.5 (90.5)	727.1 (38.9) c
Mean	946.3 (43.2) A	938.9 (66.8) A	856.3 (70.0) AB	807.4 (67.8) B	
<b>2003</b>					
0	2113.7 (45.7)	2172.0 (57.5)	2225.7 (103.2)	2154.3 (55.4)	2166.3 (31.9) a
10	2109.0 (50.7)	2092.3 (140.8)	2125.7 (76.3)	1772.0 (101.2)	2024.8 (60.8) ab
25	2028.0 (36.3)	1701.7 (114.8)	1996.7 (46.5)	1741.3 (170.2)	1874.1 (57.0) bc
50	2075.0 (189.0)	1899.3 (102.5)	1920.7 (22.8)	1668.3 (24.4)	1890.8 (63.4) bc
100	1976.7 (47.2)	1791.7 (99.9)	1787.7 (23.8)	1476.7 (84.9)	1758.2 (62.0) c
Mean	2059.4 (29.7) A	1931.4 (62.2) A	2011.3 (47.3) A	1762.5 (70.2) B	

<sup>z</sup> Means within a column followed by the same lower-case letter or within a row followed by the same upper-case letter are not significantly different according to the Tukey's studentized range test ( $P = 0.05$ ).

lower when plots were infested with bollworms for 4 wk than when plots were infested for 1 to 3 wk. When averaged across durations of infestation, yields were lower when 25 to 100% of white flowers were infested compared with the non-infested plots. Also, the 100% level of infestation resulted in lower yields than the 10% level of infestation.

**Regression analysis for bollgard cotton (2002 and 2003).** There was a significant difference in the intercepts of the regression equations relating seedcotton yields to cumulative numbers of white flowers infested for 2002 and 2003 ( $F = 957.99$ ;  $df = 1, 91$ ;  $P < 0.01$ ) This difference reflected a difference in yield potential between the two years (Fig. 1).



**Figure 1. Regression analyses for seedcotton yield of Bollgard cotton as a function of the cumulative numbers of white flowers infested per 2-m<sup>2</sup> plot. Data for 2002 and 2003 were forced to have the same slope.**

Despite the difference in yield potential, the slopes of the regression equations were similar between the 2 yr ( $F = 0.43$ ;  $df = 1, 97$ ;  $P = 0.51$ ; 2002, slope =  $-1.55 \pm 0.22$ ; 2003, slope =  $-1.80 \pm 0.27$ ). Therefore, a separate analysis was conducted where the slopes were forced to be the same to obtain a common value that could be used for predicting yield reductions by bollworms. There was a significant relationship between the numbers of white flowers infested and seedcotton yield ( $R^2 = 0.92$ ;  $F = 3277.2$ ;  $df = 3, 92$ ;  $P < 0.01$ ). The intercepts of the regressions were 1044.0 g and 2057.2 g per plot in 2002 and 2003, respectively. The common slope for the regression equations was -1.69 indicating a 1.69 g reduction in seedcotton yield for each white flower infested with a bollworm.

## DISCUSSION

White flowers provide little control of bollworms in Bollgard cotton (Gore et al. 2001). Consequently, injury to Bollgard cotton results from neonate bollworms and 1- to 2-d-old larvae feeding in white flowers and migrating to other structures when they have attained a size better able to tolerate the Cry IAc protein in Bollgard cotton (Gore et al. 2003). Injury from those larvae is mostly to bolls, and to a lesser extent, squares (Gore et al. 2003). In the current study, bollworms delayed maturity of Bollgard cotton

when 50 or 100% of white flowers were infested for at least 2 wk or reduced yields when 50 or 100% of white flowers were infested averaged across weeks in 2002 indicating the need for supplemental insecticide applications to prevent economic losses from bollworms.

During 2003, bollworms had less of an impact on Bollgard cotton. This is most likely a result of better growing conditions in 2003 than in 2002. In 2003, record cotton yields were observed across Mississippi, as well as the United States (USDA-NASS, 2003). When averaged across durations of infestation, delays in maturity were only observed when 100% of white flowers were infested, but yields were reduced when 25 to 100% of white flowers were infested. Similar to 2002, the greatest impact of bollworms on yield occurred when the duration of infestation was 4 wk. This again supports the need for applications of insecticides to Bollgard cotton under certain situations to prevent yield losses from bollworms.

Control measures for any insect pest should be initiated to prevent an increasing population from reaching a level that will cause economic losses (Pedigo et al., 1986). Therefore, insecticide applications for bollworms on Bollgard cotton should be initiated at a level before bollworms cause significant delays in maturity or yield reductions. Results of these experiments suggest insecticide applications should be made before 100% of white flowers have been infested for one week or before 25% of white flowers have been infested for 2 to 4 wk. Currently, insecticide applications are recommended when five to eight live larvae are found per 100 small bolls in Mississippi (Mississippi State University Extension Service, 2003), Georgia (Guillebeau, 2001), South Carolina (Roof, 2002), and Louisiana (Bagwell et al., 2002). Based on results from this study and expected survival rates of bollworms on white flowers and small bolls (Gore et al., 2003), these action levels appear to be conservative, but appropriate for Bollgard cotton to prevent economic losses from bollworms. Gore et al. (2003) found that approximately 25% of neonates in white flowers survive and damage the small boll that develops at that fruiting site.

Future research will be needed to determine if current thresholds are too conservative. Based on the slope of the regression equation for numbers of white flowers infested and seedcotton yield, a 1.69-g reduction in seedcotton can be expected for every white flower infested with a bollworm. This equates

to a 1-kg loss for every 592 white flowers infested (1-lb. loss for every 269 white flowers). Future research will need to be conducted to determine the most appropriate method to scout Bollgard cotton for bollworms in white flowers. Once an appropriate method has been adopted for estimating the numbers of white flowers per hectare, our regression equation can be used to estimate yield losses on individual fields. With this information, agricultural consultants and pest management professionals will be able to efficiently and accurately determine the need for insecticide applications targeting bollworms in Bollgard cotton.

### ACKNOWLEDGEMENT

The authors thank Don Hubbard, Leslie Price, and numerous student employees for technical assistance and Drs. Carlos Blanco, William Meridith, and Sam Turnipseed for critical reviews of this manuscript.

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