## THE "HALO" EFFECT ASSOCIATED WITH BOLLGARD COTTON REFUGES R.H. Gable and B.R. Leonard LSU AgCenter, Macon Ridge Research Station Winnsboro, LA D.R. Cook and M.M. Willrich LSU AgCenter, Department of Entomology Baton Rouge, LA J. Gore USDA-ARS SIMRU Stoneville, MS

#### **Abstract**

A field test was conducted to evaluate the influence of non-Bollgard cotton refuges on heliothine damage in 2001 and 2002 at the LSU AgCenter's Macon Ridge location of the Northeast Research Station. Embedded non-Bollgard refuge sizes of 48, 24, and 16-rows wide by 160 ft in length were evaluated. In both years the test was sampled after defoliation and before harvest. Heliothine damaged bolls decreased and seedcotton yield increased in Bollgard<sup>®</sup> cotton as distance increased from a non-Bollgard<sup>®</sup> cotton refuge. There were 7% and 20% damaged bolls in the refuge and 0.9% and 1.1% in the Bollgard cotton for the 48-row regime in 2001 and 2002, respectively. For the 24-row regime, 5% and 23% damaged bolls were observed in the refuge during 2001 and 2002, respectively. Damaged bolls in Bollgard<sup>®</sup> cotton of the 24-row regime were 0.7% and 2% in 2001 and 2002, respectively. There were 12% and 20% damaged bolls in the 16-row refuge regime and 4% and 2% in the associated Bollgard cotton during 2001 and 2002, respectively. Lint yield for the refuge of 48-rows was 100 and 350 lb/acre higher in the Bollgard<sup>®</sup> cotton than the associated refuge in 2001 and 2002, respectively. Estimates of late-season heliothine damaged bolls suggest non-Bollgard cotton refuges are successful in allowing heliothines to develop to late instar stages and potentially add to the local adult populations.

#### **Introduction**

Bollgard<sup>®</sup> is a major component in cotton insect pest management programs. Bollgard<sup>®</sup> cotton provides an environmentally friendly and economical alternative to insecticide-based crop protection strategies, without sacrificing yield. Bollgard<sup>®</sup> or Bt cotton, produces the Cry1Ac protein derived from the soil bacterium *Bacillus thuringiensis* (Bt) Berliner var. *kurstaki.* (Greenplate et al. 2001). This protein is toxic to larval stages of specific lepidopteran pests (Perlak et al. 1990, Stewart et al. 2001). Bollgard<sup>®</sup> cotton exhibits excellent insecticidal activity against tobacco budworm, *Heliothis virescens* (F.), and pink bollworm, *Pectinophora gossypiella* (Saunders) (Stewart et al. 2001). However, Bollgard<sup>®</sup> has limited activity against bollworm, *Helicoverpa zea* (Boddie); armyworms, *Spodoptera* spp.; and soybean loopers, *Pseudoplusia includens* (Walker) (Luttrell et al. 1999). Bollgard<sup>®</sup> cotton requires supplemental insecticide applications to prevent economic injury from persistent populations of non-target pests. (Bacheler and Mott 1997, Leonard et al. 1997, and Gore et al. 2001).

In laboratory studies, some caterpillar pests have demonstrated the ability to become insensitive to the Bt protein (Gould and Tabashnik 1998). Insect resistance management (IRM) practices are aimed at reducing the development of resistance by conserving the pest's susceptibility. IRM practices in Bollgard<sup>®</sup> cotton should include the knowledge of the pest's biology and ecology, an appropriate expression level of the Bt protein to kill all heterozygotes, the design and deployment of a non-Bt refuge, and a heliothine susceptibility monitoring (Bt) program.

One of these components managed at the farm level is implementation of the refuge strategy. A refuge is an area of non-Bt cotton planted in close proximity to Bollgard<sup>®</sup> cotton. In 2002, the three refuge options included: 1) a 95:5 external non-sprayed refuge (for every 95 acres of Bollard<sup>®</sup> cotton 5 acres of non-Bt cotton must be planted. The refuge must be 150 ft wide and cannot be treated with any insecticide recommended for heliothines), 2) an 80:20 external sprayed refuge (for every 100 acres of Bollard<sup>®</sup> cotton 20 acres of non-Bt cotton must be planted), and 3) a 95:5 embedded refuge (a new option in which the non-Bollgard<sup>®</sup> variety can be planted in a contiguous block within the Bollgard<sup>®</sup> field. For every 95 acres of Bollgard<sup>®</sup> cotton 5 acres of non-Bt producing cotton must be planted). This refuge can be sprayed with any lepidopteran active insecticide as long as the entire Bollgard<sup>®</sup> field is sprayed at the same time. The refuge for the 80:20 and 95:5 non-sprayed option should be planted within one mile of the Bollgard<sup>®</sup> cotton. In Louisiana, the most popular refuge structures are the 80:20 sprayed and the 95:5 embedded Bollgard<sup>®</sup> and non-Bollgard<sup>®</sup> cultivars, respectively. The objective of the refuge is to maintain a Bt susceptible insect population. In the event, a Bt resistant moth survives and emerges from Bollgard<sup>®</sup> cotton, the adjacent refuge supports a susceptible moth population that can mate with the resistant moth reducing the potential development of a Bt resistant population.

The design and deployment of refuges for Bollgard<sup>®</sup> cotton are important IRM considerations. Immigration patterns of pest adults should be considered when determing the design and placement of a refuge (Matten 2001). Gould and Tabashnik (1998) recommended that to maintain an appropriate spatial scale, 50% of the cotton acreage be planted to non-Bt when refuges are to be sprayed with lepidopteran active insecticides and 16.7% of the cotton acreage be planted to non-Bt cotton when the refuges are not to be treated for heliothines. Refuges should be maintained using the same agronomic practices as the Bollgard<sup>®</sup> cotton (fertilizer, herbicide, irrigation, etc.) to prevent asynchronous crop development.

A general phenomenon termed the "halo" effect has been observed in non-Bt cornfields planted adjacent to cornfields producing the Bt protein. In general, a reduction in insect injury in fields of non-Bt corn planted adjacent to fields of Bt corn has been observed. A decrease (30 to 50%) in 2<sup>nd</sup> generation European corn borer, *Ostrinia nubilalis* (Hübner), injury was observed up to 150 feet away from Bt cornfields (Andow and Hutchinson 1998). This concept has not been studied for Bt (Bollgard<sup>®</sup>) cotton and may have considerations for non-Bollgard<sup>®</sup> cotton refuges. The "halo" surrounding Bt fields may reduce the number of heliothine damaged bolls in the refuge and influence heliothine injury in adjacent Bollgard<sup>®</sup> cotton. The objective of this study is to determine halo effects on damaged bolls and seedcotton yield in Bollgard<sup>®</sup> cotton and associated non-Bollgard cotton refuge. A better understanding of the halo effect could influence how cotton refuges are deployed in the future.

## Materials and Methods

A field test was conducted during 2001 and 2002 at the LSU AgCenter's Macon Ridge location of the Northeast Research Station, using the 95:5 embedded refuge configuration. Embedded non-Bollgard refuges were configured in sizes of 48, 24, and 16-rows wide by 160 ft in length were evaluated. In 2001, Stoneville 580 cotton seed was utilized in the non-Bollgard refuges and Delta Pine 428B was used as the Bollgard<sup>®</sup> cultivar. In 2002, Stoneville 4793R cotton seed was utilized in the non-Bollgard refuges and Stoneville 4892BR for the Bollgard<sup>®</sup> cultivar. Refuge areas and Bollgard<sup>®</sup> fields were planted on 28 May and 27 May in 2001 and 2002, respectively. To maintain naturally high Heliothine densities, the test area did not receive a lepidopteran-active insecticide.

## Insect Damage

After cotton plots were defoliated, the test areas were sampled for late-season heliothine damaged bolls in October and November during 2001 and 2002, respectively. The specific rows used for monitoring insect damage in the refuge and adjacent Bollgard cotton was determined prior to the initial sample. This pattern was used on each monitoring row (Fig. 1, 2, 3). Each sample site was replicated three times within the refuge and Bollgard<sup>®</sup> areas. All bolls (closed and open) were examined within a 1-meter section of row for larval injury. A boll was considered damaged when a hole through the carpel wall could be attributed to caterpillar feeding.

## Seedcotton Yield

Selected rows were harvested within the 48-row non-Bollgard cotton refuge and across the associated Bollgard<sup>®</sup> cotton with a mechanical picker to determine seedcotton yield (Fig. 4). The sample sites were harvested on 19 October and 14 November in 2001 and 2002, respectively. Seedcotton was converted to lint yield based on the cultivar's lint:seed ra-tio.Results and Discussion.

## Refuge of 48 Rows

In 2001, the mean number of heliothine damaged bolls ranged from 5% to 11% in the refuge (Fig. 5). The greatest percentage of damaged bolls (1.4%) was observed on the first row of Bollgard<sup>®</sup> cotton adjacent to the refuge and declined to 0.27% as sample sites moved further away from the refuge. In 2002, the mean number of damaged bolls in the refuge ranged from 15% to 24%. There were 19% damaged bolls on the first row of refuge compared to 0.42% on the first row of Bollgard<sup>®</sup> cotton. Damaged bolls in the Bollgard cotton ranged from 0.4% to 2% (Fig. 5).

## **Refuge of 24 Rows**

In 2001, damaged bolls in the refuge ranged from 3.1% to 8.5% (Fig. 6). In the Bollgard<sup>®</sup> cotton, the highest number of damaged bolls was recorded on the row adjacent to the refuge (1.5%). The percentage of damaged bolls decreased across the Bollgard<sup>®</sup> cotton with 0% recorded 6 rows away from the refuge. In 2002, the mean number of damaged bolls in the refuge ranged from 16% to 23% (Fig. 6). In the Bollgard cotton, the mean number of damaged bolls ranged from 0.6% to 2.6%.

## **Refuge of 16 Rows**

In 2001, the percentage of damaged bolls in the refuge ranged from 8.6% to 14.8% (Fig. 7). In the Bollgard<sup>®</sup> cotton, the highest number of damaged bolls (5.4%) was observed on the row adjacent to the refuge. In 2002, damaged bolls in the refuge ranged from 18% to 22.5% (Fig. 7). In the Bollgard cotton damage ranged from 0.6% to 3.7%. The Bollgard cotton row adjacent to the refuge had 2.4% of bolls damaged on the row adjacent to the refuge, compared to 18.9% on the first row of the refuge.

# Seedcotton Yield

Lint yield in the non-Bollgard refuges averaged 581 and 209 lb/acre during 2001 and 2002, respectively, while yield in the Bollgard<sup>®</sup> cotton averaged and 681 to 565 lb/acre during 2001 and 2002, respectively (Fig. 8). In 2001, the lint yield in the refuge ranged from 557 to 605 lb/acre and 641 to 774 lb/acre in the Bollgard<sup>®</sup> cotton. In 2002, lint yield in the refuge ranged from 150 to 268 lb/acre and 432 to 648 lb/acre in the Bollgard<sup>®</sup> cotton.

#### **Summary**

Bollworm populations were higher in 2002 compared to 2001 resulting in more damaged bolls in the refuge. Higher numbers of damaged bolls were generally observed in the refuge than in the Bollgard<sup>®</sup> cotton in both years. Numbers of heliothinedamaged bolls were 4 and 11.2 fold higher in the non-Bollgard cotton refuges compared to the Bollgard cotton during 2001 and 2002, respectively. Boll damage fluctuated within the refuge with lower numbers of damaged bolls recorded on non-Bollgard plants near the Bollgard:refuge interface. The number of bolls damaged by heliothine larvae was generally greater within the first 10 rows of Bollgard compared to the sample sites further away from the refuge. The number of damaged bolls fluctuated as sample sites moved further away from the refuge. Seedcotton yield varied among refuge sample sites during each year. In 2001, seedcotton yield in the refuge interface. In 2002, seedcotton yield in the refuge was greater on the rows harvested adjacent to the Bollgard:refuge interface. In 2002, seedcotton yield in the refuge was greater on the rows harvested next to the Bollgard: refuge interface compared to 12 rows away from the interface. Yields were generally higher in the Bollgard<sup>®</sup> compared to the refuge as sample sites were harvested further away from the Bollgard:refuge interface. Seedcotton yield in the Bollgard:refuge interface interface. Seedcotton yield in the Bollgard:refuge interface compared to 12 rows away from the Bollgard:refuge interface.

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	Bollgard Cotton															
(Sam 26,25 ●	pled ro 22,21 ↔	• w s) 18,17 ●	14,13 ●	10,9 ●	6,5 ●	2,1 ●	1 ,2 ●	5,6 ●	9,10	15,16 ●	23,24	33,34 ●	63,64 ●	93,94 ●	1 2 3 ,1 2 4 ●	153,154
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Figure 1. Sites used for monitoring heliothine damage in refuge of 48 rows and the Bollgard cotton.



Figure 2. Sites used for monitoring heliothine damage in refuge of 24 rows and the Bollgard.

	Refuge Co	tto	n	-	Bollgard Cotton										
(Sampled rows)															
	6,5	4,3	2 , 1	1 ,2	5,6	9,10	15,16	23,24	33,34	63,64	93,94	123,124	153,154		
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Figure 3. Sites used for monitoring heliothine damage in the refuge of 16 rows and Bollgard cotton.



Figure 4. Rows used to determine seedcotton yield in the non-Bollgard refuge and Bollgard cotton.



Figure 5. Spatial variation of heliothine damaged bolls per row meter, refuge of 48 rows – 2001 and 2002.



Figure 6. Spatial variation of heliothine damaged bolls per row meter, refuge of 24 rows -2001 and 2002.



Figure 7. Spatial variation of heliothine damaged bolls per row meter, refuge of 16 rows – 2001 and 2002.



Figure 8. Spatial variation in yield, in the non-Bollgard refuge and Bollgard cotton – 2001 and 2002.