DISPERSAL OF BOLLWORM LARVAE ON BOLLGARD® AND NON-BOLLGARD COTTON CULTIVARS

J. Gore, B. R. Leonard, D. R. Cook and R. H. Jones
Louisiana State University Agricultural Center
Baton Rouge, LA

Abstract

Reports of bollworm, *Helicoverpa zea* (Boddie), larvae feeding in white flowers of Bollgard® cotton have been relatively common each year since its commercialization. Currently, no information is available explaining the mechanisms that lead to bollworm infestations in white flowers. Field studies were conducted in northeast Louisiana to determine if differences in bollworm larval behavior occur on conventional (cv. Deltapine 5415) and Bollgard® (cv. NuCOTN 33B) cottons. Larvae were placed in the terminals of either single cotton plants or on all plants within 1-m row micro-plots. On non-flowering cotton plants, significantly more bollworms moved from the site of infestation (terminal) on Bollgard plants compared to that on non-Bollgard plants. On individual flowering plants, the number of nodes larvae moved from the terminal and number of infested bolls were greater on Bollgard cotton plants. Similar differences between Bollgard and non-Bollgard plants in the percentage of infested terminals and squares were observed at 48-h after infestation when 1-m rows were infested. These data will be used to refine scouting protocols for bollworm larvae on Bollgard® cotton.

Introduction

Genetically modified plants are an important component of integrated pest management (IPM) programs in many cropping systems. Bollgard® cotton cultivars that express the Cry1Ac protein from the soil bacterium, *Bacillus thuringiensis kurstaki* Berliner, (Perlak et al. 1990) have become cost effective and environmentally friendly tools for selective pest management. Bollgard cotton was introduced for commercial production in 1996, and since that time the acreage planted to these cultivars has increased every year in most states. Bollgard cotton provides excellent control of the tobacco budworm, *Heliothis virescens* (F.), and pink bollworm, *Pectinophora gossypiella* (Saunders) (MacIntosh et al. 1990, Luttrell et al. 1999). Bollworms, *Helicoverpa zea* (Boddie), also are susceptible to the Cry1Ac protein (MacIntosh et al. 1990, Luttrell et al. 1999). Bollgard provides satisfactory control against low to moderate bollworm densities. However, insecticide applications are often needed to prevent economic injury when high population densities persist for several days (Bachelor and Mott 1997; Layton et al. 1997, 1998; Leonard et al. 1997, 1998; Roof and DuRant 1997; Smith 1997, 1998).

White flowers appear to be the plant structures where bollworm larvae are most often observed feeding (Smith 1998, Pietrantonio and Heinz 1999). During 1996, bollworm populations were extremely high in most areas of the mid-southern U. S., southeastern U. S., and Texas. Consequently, crop advisors in those regions observed the presence of large numbers of bollworm larvae in Bollgard cotton fields. The majority of these populations consisted of small larvae (≤L2) feeding within white flowers. Currently, there is little information explaining why bollworms are more commonly found in white flowers of Bollgard cotton than non-Bollgard cotton. One proposed theory is that bollworm oviposition is different on Bollgard plants compared with non-Bollgard plants and that more eggs are deposited lower in the plant canopy on Bollgard cotton. Differences in sites of oviposition would not be expected between Bollgard cotton and non-Bollgard cotton since the Cry1Ac protein in Bollgard cotton should not affect bollworm adults (MacIntosh et al. 1990). Furthermore, the morphology of Bollgard cottons should be similar to the parental non-Bollgard breeding lines. Parker and Luttrell (1998) found no differences in tobacco budworm egg density on Bollgard cottons compared with the non-Bollgard parental cottons. Also, the vertical distribution of eggs on plants was not different between Bollgard and non-Bollgard plants (Parker and Luttrell 1998). In Louisiana, no differences were observed in the number of soybean looper, *Pseudoplusia includens* (Walker), eggs recovered from a Bollgard cultivar and a non-Bollgard cotton cultivar (Hall 2000).

An alternative theory is that early instar larval dispersal is different on Bollgard cotton plants compared to non-Bollgard cotton plants. Tobacco budworm larval movement has been observed to be different on Bollgard cotton plants compared to non-Bollgard plants in field and greenhouse studies (Benedict et al. 1993, Parker and Luttrell 1999). In both of these studies, tobacco budworm larvae moved from Bollgard plant terminals faster than on non-Bollgard plants. These authors did not report on the fate of larvae after leaving the terminals. Larvae are the developmental stage controlled by the Cry1Ac protein in Bollgard cotton, and differences in larval behavior could result in feeding preferences on specific plant parts. Therefore, studies were conducted in Louisiana to determine if differences in bollworm larval behavior occur on Bollgard cotton plants compared to non-Bollgard plants. This study consisted of one experiment during vegetative plant development and two experiments during reproductive plant development.

Materials and Methods

Blocks (16 rows x 100 ft.) of a Bollgard cotton cultivar (NuCOTN 33B) and a non-Bollgard parental cultivar (Deltapine 5415) were planted at the Macon Ridge location of the Northeast Research Station near Winnsboro, LA in 1999 and 2000. Fertilization rates and general agronomic practices for cotton production followed current Louisiana Cooperative Extension Service recommendations.

Bollworms were collected from clover, *Trifolium* spp., during April and sweet corn, *Zea mays* L., (cv. SG 90) during June. Colonies were maintained in the laboratory for at least one generation to eliminate parasitoids, minimize pathogens, and obtain sufficient numbers of larvae at the proper stage for infestations on cotton plants. Larvae were fed a wheat germ/soy protein diet (*Heliothis* premix, Stonefly Industries, Bryan, TX) until pupation. Adults were held in 3.79-L cardboard containers and fed a 10% sugar-water solution. A single layer of cheesecloth was placed over the containers to provide an adequate surface for moth oviposition. Egg sheets were harvested daily and placed into plastic bags until larval eclosion. Upon eclosion, larvae were fed meridic diet in 236-ml cups (ca. 50 larvae/cup) for ca. 48-h. After 48±3-h, bollworm larvae were infested on cotton plants during vegetative or reproductive developmental stages. Larvae were placed in cotton plant terminals using a small paintbrush.

Infestation of Individual Pre-Flowering Cotton Plants

Bollgard and non-Bollgard cotton plants were infested with a single bollworm larva during pre-flowering growth stages to determine if differences in movement occur. Individual plants were thinned prior to infestation so that no interplant movement could occur. A 40.6-cm x 40.6-cm sticky trap was placed at the base of each infested plant. Sticky traps were used to recover larvae that apparently left plants by "spinning-down" on a silken thread. This experiment consisted of six replications (over time) in a randomized complete block design. Blocks were represented by day of infestation and at least 20 plants were infested per day. Number of larvae found on sticky traps was recorded at 1-h after infestation (HAI), 3 HAI, 6 HAI, and 24 HAI. Data were converted to percentages and comparisons were made between Bollgard cotton and non-Bollgard cotton using paired t-tests.

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Infestation of Individual Flowering Cotton Plants

First instar bollworm larvae were infested on individual cotton plants (one larva/plant) during flowering growth stages. Individual plants were thinned prior to infestation so that no interplant movement could occur. Procedures and experimental design for larval infestations were similar to those described for pre-flowering cotton plants except sticky traps were not used. Bollworm infested plants were examined at 3 HAI, 6 HAI, and 24 HAI. Numbers of main stem nodes a larva moved from the plant terminal and numbers of fruiting structures (square, flower, boll) infested with a larva were recorded. Data were compared between Bollgard cotton and non-Bollgard cotton using the Wilcoxon rank-sum test (PROC NPAR1WAY, SAS Institute 1989).

Infestation of Multiple (1-m Row) Flowering Cotton Plants

Micro-plots (1 row x 1-m) were established in large blocks of Bollgard and non-Bollgard cotton cultivars. Plants in micro-plots were infested with 20 first instar bollworm larvae. Larvae were placed in the terminals of plants using a small paintbrush. A total of 20 micro-plots were infested for non-Bollgard and Bollgard cotton. The experimental design was a randomized complete block and dates of infestation represented blocks. Whole plants within each micro-plot were inspected at 24 HAI and 48 HAI. Plant, square, flower, and boll densities were recorded from each micro-plot. Numbers of plant terminals, squares, flowers, and bolls infested with larvae were recorded. Data were converted to percentages and each variable was compared between Bollgard and non-Bollgard cotton using the Wilcoxon rank-sum test (PROC NPAR1WAY, SAS Institute 1989).

Results

Bollworm Movement on Individual Pre-Flowering Cotton Plants

Higher percentages of bollworm larvae were observed on sticky traps beneath Bollgard plants compared to traps beneath non-Bollgard plants at all rating intervals (Fig. 1). At 1 HAI, 17% of the total number of larvae infested on plants were recovered on sticky traps beneath Bollgard plants compared to 7% beneath non-Bollgard plants (t=-3.27, df=22.0, P<0.01). At 3 HAI, 40% of the total number of bollworm larvae infested on Bollgard plants were found on sticky traps compared to 9% on non-Bollgard plants (t=3.99, df=18.0, P<0.01). At 6 HAI, 47% and 11% of the total number of larvae were recovered from sticky traps beneath Bollgard and non-Bollgard cotton plants, respectively (t=5.19, df=18.0, P<0.01). At 24 HAI, 49% of infested larvae were recovered from sticky traps beneath Bollgard cotton plants compared to 12% beneath non-Bollgard cotton plants (t=5.45, df=18.0, P<0.01).

Bollworm Movement on Individual Flowering Cotton Plants

Similar to the results for pre-flowering cotton, bollworm larvae moved significantly more on Bollgard plants compared to non-Bollgard plants. Bollworm larvae were found 4.25 main stem nodes below plant terminals on Bollgard cotton compared to 2.48 main stem nodes below plant terminals on non-Bollgard cotton at 6 HAI (P=0.03) (Fig. 2). At 24 HAI, larvae were found an average of 5.70 main stem nodes below the terminals on Bollgard plants compared to 2.93 main stem nodes below the terminals on non-Bollgard cotton (P=0.01).

No significant differences in the numbers of infested terminals, squares, or bolls were observed between Bollgard cotton and non-Bollgard cotton at 6 HAI (Fig. 3). No larvae were found in non-Bollgard flowers; whereas, 1.75 larvae were found in Bollgard flowers. . At 24 HAI, significantly more larvae were found in Bollgard cotton bolls (4.75) compared to non-Bollgard cotton bolls (1.00) (P=0.01) (Fig. 4). However, significantly fewer larvae were found in Bollgard squares (3.25) compared to non-Bollgard cotton squares (7.50) at 24 HAI (P=0.04).

Bollworm Movement on Multiple (1-m row) Flowering Cotton Plants

Numbers of plants, squares, flowers, and bolls ranged from 5 to 10, 56 to 116, 0 to 6, and 24 to 53, respectively, within Bollgard and non-Bollgard micro-plots during the infestation period. Fewer bollworm larvae remained in plant terminals of Bollgard cotton (1.98%) compared to that of non-Bollgard cotton plants (31.61%) at 24 HAI (P<0.01) (Fig. 5). In addition, a significantly higher percentage of bolls were infested in Bollgard cotton (4.69%) compared to non-Bollgard cotton (0.68%) (P=0.05). At 24 HAI, 1.7% of Bollgard cotton flowers were infested with bollworm larvae. No larvae were found in non-Bollgard flowers; whereas, 2.5% of white flowers were infested with bollworm larvae. At 48 HAI, significantly fewer larvae were found in plant terminals (P<0.01) and squares (P<0.01) on Bollgard cotton compared to those structures on non-Bollgard cotton (Fig. 6). Larvae remaining in plant terminals averaged 1.21% on Bollgard cotton and 12.21% on non-Bollgard cotton. The percentage of bollworm larvae found in squares averaged 0.80% on Bollgard cotton compared to 3.23% on non-Bollgard cotton. A significantly higher percentage of white flowers were infested on Bollgard cotton (9.67%) compared to flowers of non-Bollgard cotton (2.22%) (P=0.02).

Discussion

Cotton pest management consultants have experienced difficulties in making decisions about when to apply foliar insecticides to manage bollworms in Bollgard cotton. Most current sampling plans for non-Bollgard cotton are based on larvae in plant terminals. Large numbers of bollworm larvae have been observed in white flowers of Bollgard cotton every year since its introduction in 1996. The data in the present study indicate that bollworm larvae disperse more rapidly on Bollgard cotton compared to non-Bollgard cotton. Bollworm larvae moved 2.90 nodes below Bollgard plant terminals within 3 HAI, but only moved 2.48 nodes within 6 HAI on non-Bollgard plants. Also, those larvae moved a greater vertical distance on Bollgard cotton. Bollworm moths typically utilize the top one third of plants for oviposition (Farrar and Bradley 1985). Therefore, the majority of eggs are usually found in or near plant terminals (Wilson et al. 1980). Small bollworm larvae remain near the terminals of non-Bollgard cotton plants feeding on small squares. Fye (1972) found that 78 to 100% of damaged fruiting forms could be found in the top 0.6-m of plants at any given time. As larvae develop, they typically move down the plants feeding on larger squares and bolls (Wilson and Gutierrez 1980). In the present study, larvae remained near the top of non-Bollgard cotton plants feeding on terminal foliage and small squares. In contrast, larvae were observed lower in the plant canopy on Bollgard cotton feeding on white flowers and bolls.

Currently, action thresholds to initiate heliothine (bollworm/tobacco budworm) control with foliar sprays are based on numbers of eggs and/or larvae in terminals, and numbers of larval infested/damaged squares on non-Bollgard cotton. In Louisiana, insecticide applications are recommended when at least 5 live larvae per 100 plants plus eggs are present (Bagwell et al. 2000). These thresholds and scouting methods are not appropriate for Bollgard cotton, because larvae feeding on white flowers and bolls may be missed. For the 1-m row infestations, the percentage of infested terminals averaged 12.2% on non-Bollgard cotton at 48 HAI. This level is above the current action threshold and the non-Bollgard plots would be treated with foliar insecticide applications. Also, 3.2% of non-Bollgard squares were infested with larvae. In contrast, 1.2% and 0.8% of Bollgard terminals and squares were infested with larvae, respectively, within 48 HAI. Based on current action thresholds, Bollgard cotton would not require treatment. However, if the percentages of infested flowers (9.7%) and bolls (4.2%) are also considered, Bollgard cotton may require insecticide applications to prevent economic yield loss.

In addition, bollworm larvae began moving out of plant terminals within 1 HAI on Bollgard cotton. Therefore, when eggs hatch, there is a narrow period of time when larvae can still be observed in or near plant terminals. In the vegetative study, over 50% of larvae that were originally infested on pre-flowering plants migrated away from plant terminals within 6 HAI. Field scouts searching for bollworm infestations in plant terminals are likely not to find larvae in the terminals when sampling during more than 6 hours after larval eclosion.

These data suggest that current scouting protocols and action levels to initiate insecticide treatments for bollworms on non-Bollgard cotton are not appropriate for Bollgard cotton. Scouts should look at white flowers and small bolls in addition to terminals and squares when scouting Bollgard cotton. More data are needed to determine the percentage of small larvae feeding on fruiting structures low in the plant canopy that are capable of causing economic injury. This information will be necessary to further refine action thresholds for bollworms in Bollgard cotton.

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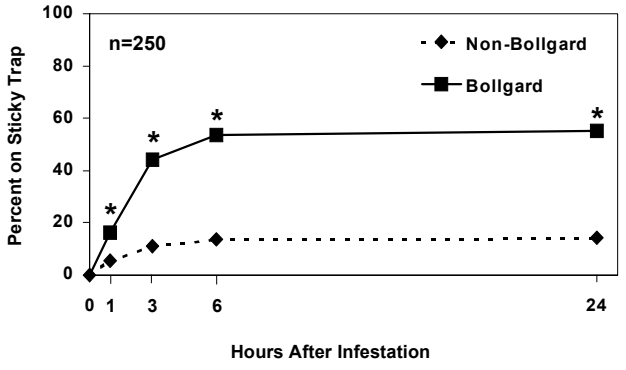

Figure 1. Bollworm larval movement off cotton plants during pre-flowering growth stages (* indicate significant differences between Bollgard cotton and non-Bollgard cotton).

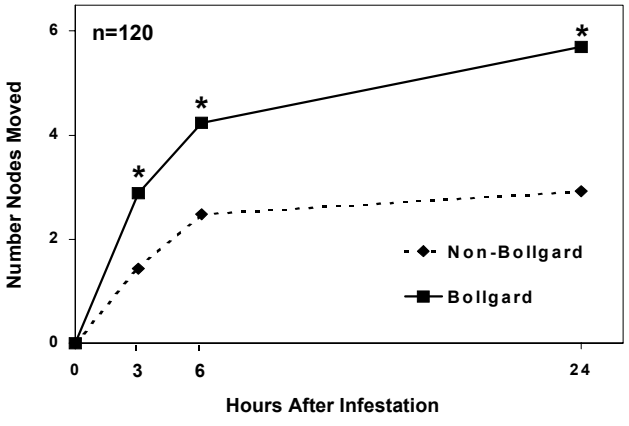

Figure 2. Bollworm larval movement on cotton plants during flowering stages of cotton plant development (* indicate significant differences between Bollgard cotton and non-Bollgard cotton).

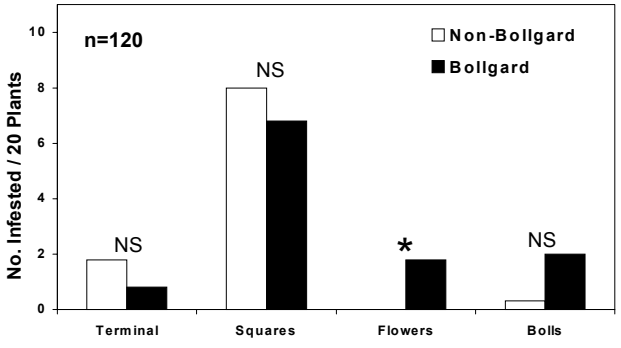

Figure 3. Bollworm larval movement (6 HAI) on individual cotton plants during flowering stages of cotton plant development (NS = not significant; * no larvae were found in non-Bollgard white flowers).

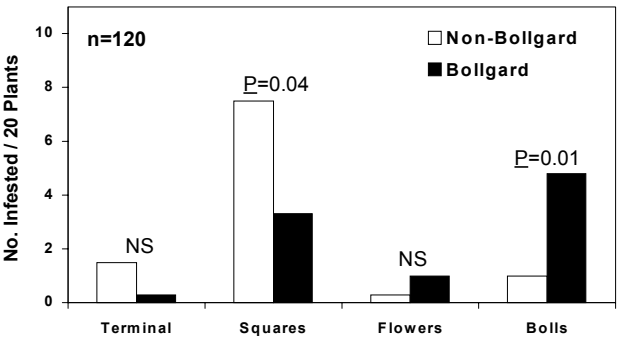

Figure 4. Bollworm larval movement (24 HAI) on individual cotton plants during flowering stages of cotton plant development (NS = not significant).

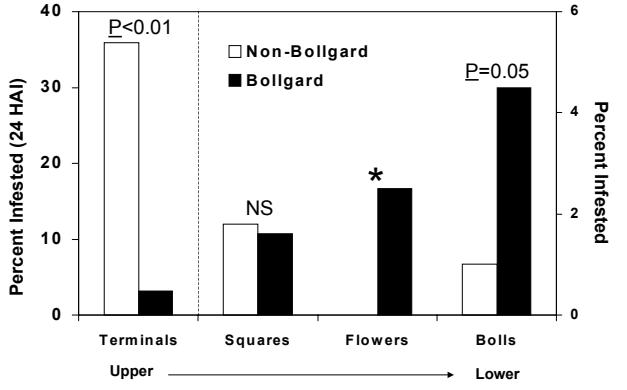

Figure 5. Bollworm larval dispersal (24 HAI) on 1-m rows of cotton during reproductive growth stages (NS = not significant; * no larvae were found in non-Bollgard white flowers).

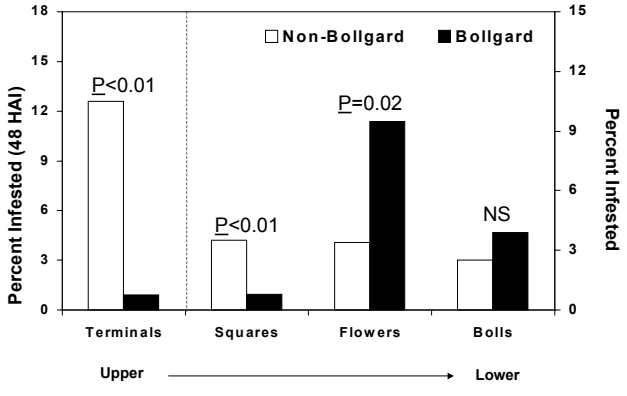

Figure 6. Bollworm larval dispersal (24 HAI) on 1-m rows of cotton during reproductive growth stages (NS = not significant).