2004 BELTWIDE COTTON CONFERENCES, SAN ANTONIO, TX – JANUARY 5-9

DESCRIBING THE MICRO- AND MACROSCALE MOVEMENT OF HELIOTHINES IN BOLLGARD COTTON SYSTEMS

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Abstract

A field trial evaluated the interplant movement of tobacco budworm, *Heliothis virescens* (F.), larvae on Bollgard 2 cotton. A small plot study was conducted at the Macon Ridge Research Station using three row microplots. Treatments included Stoneville 4563B2 (Bollgard 2) planted on all three rows, Bollgard 2 planted on the center row with Stoneville 474 (conventional cultivar) planted on each side, and conventional cultivar planted all three rows. Fourth instar tobacco budworm larvae were infested on the center row of each treatment. There were significantly fewer damaged and infested fruiting forms on the Bollgard 2 plants compared to the conventional cotton. Yields in both BG2 plots were significantly higher than that in the conventional cotton plots. In a second study, Texas type 75-50 wire cone traps were utilized to determine the spatial distribution of heliothine moths adjacent to a cotton refuge. Traps for bollworm, *Helicoverpa zea* (Boddie), and tobacco budworms were placed at the center of the refuge and at half mile increments, for a distance of two miles, away from the refuge. Traps were sampled weekly between Jun and mid Oct. The total number of bollworm moths ranged from 2199 to 3821, and averaged 3422 moths per site. The fewest number of bollworms were collected in the refuge and densities increased at sites farther away from the refuge. The total number of tobacco budworm moths ranged from 818 to 136, among trap sites. The average capture at each site was 426 moths. The highest number of tobacco budworm moths was collected within a half mile of the refuge border. Densities declined at trap sites farther away from the refuge.

Introduction

Bollgard cotton is a major component of cotton IPM. Bollgard cotton provides an environmentally friendly and economical alternative to insecticide-based crop protection strategies, without sacrificing yield. Bollgard, 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 cotton exhibits excellent insecticidal activity against tobacco budworm, *Heliothis virescens* (F.), and pink bollworm, *Pectinophora gossypiella* (Saunders) (Stewart et al. 2001). However, Bollgard has limited activity against bollworm, *Helicoverpa zea* (Boddie); armyworms, *Spodoptera* spp.; and soybean loopers, *Pseudoplusia includens* (Walker) (Luttrell et al. 1999). Bollgard 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).

Monsanto has developed a second generation of transgenic insect resistant cotton products, Bollgard 2, with higher efficacy against bollworms. Bollgard 2 also has activity against other lepidopteran larvae, and is included in insect resistant management (IRM) plans to reduce the potential for resistance to the Cry1Ac protein in Bollgard. Bollgard 2 expresses both the Cry1Ac and the Cry2Ab proteins (Greenplate et al. 2000). These plants exhibit a 10-fold increase of Cry2Ab above the level of Cry1Ac and have resulted in a 3.5-fold decrease in survival rates of selected lepidopteran larvae (Greenplate et al. 2000). Bollgard 2 gained U.S. regulatory approval in 2003. The single gene Bollgard varieties will likely be phased out in favor of the two gene varieties over the next several years (Voth et al. 2001).

In laboratory studies, some caterpillar pests have demonstrated the ability to become insensitive to Bt proteins (Gould and Tabashnik 1998). Insect resistance management (IRM) practices are aimed at reducing the development of resistance by conserving the pest's susceptibility to Bt. IRM practices in Bollgard 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 (Bt) monitoring program.

The design and deployment of refuges for Bollgard cotton are important IRM considerations. Immigration patterns of pest adults should be considered when determining 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 should be planted to non-Bt when refuges are to be sprayed with lepidopteran active insecticides and 16.7% of the cotton acreage should 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 cotton (fertilizer, herbicide, irrigation, etc.) to prevent asynchronous crop development.

Studies indicate the evolution rate of resistant genotypes may occur at a higher frequency in an embedded refuge than in an isolated refuge. Mallet and Porter (1992), using computer models, suggested resistant populations would increase when there is interplant larval movement from non-Bollgard to Bollgard cottons. Parker (1997) infested tobacco budworm larvae, at different stages of development, on BG cotton containing the Cry1Ac and conventional cotton. Parker (1997) reported that tobacco budworm larvae, when infested on Bollgard cotton planted adjacent to conventional cotton, moved away from transgenic plants more than when infested on conventional cotton. An average of 85 percent of the surviving larvae will move at least one plant by the age of 10-days-old for larvae infested on Bollgard cotton adjacent to conventional cotton. Gore (2003) reported that bollworm larvae, when infested in the terminal area of Bollgard cotton, began migrating out of the terminal within three hours after infestation. Most of the larvae found were observed feeding on white flowers and young bolls. Benedict et al. (1993) reported that tobacco budworm larvae, when infested on Bollgard and conventional cottons, spent more time at rest and less time feeding on Bollgard cotton than on conventional cotton. There is no quantitative field data to describe interplant movement of tobacco budworm larvae on Bollgard 2 cotton. A field test was conducted to determine if tobacco budworm larvae could survive in pure and mixed planted plots of Bollgard 2 and conventional cotton and damage fruiting structures on adjacent rows.

Another important IRM question concerns the density of bollworm and tobacco budworm moths produced from a conventional refuge and the distance adults will migrate from that refuge. Pheromone baited wire-cone traps have been an effective method at monitoring adult heliothine populations. Using pheromone traps Schneider (2003) studied the sources of adult bollworm and tobacco budworm populations in the early spring. The spatial distribution of heliothine adults in a Bollgard cotton system has not been clearly defined. Therefore, the second objective of this project was to determine spatial densities of heliothine moths in a cotton refuge and its associated Bollgard cotton fields.

Materials and Methods

Microplot Study

Field plots were established by planting cottonseed on 9 Jun in three rows plots (40-inch centers) X 25 ft. at the LSU AgCenter's Macon Ridge Research Station. The three treatments consisted of different combinations of three planting units with the center row being designated as the infested row. The three planting units were: 1) Stoneville 4563B2 [BG2] in the center with Bollgard 2 on each side (BG2-BG2-BG2), 2) Bollgard 2 on the center row and Stoneville 474 [CVT] on each side (CVT-BG2-CVT), and 3) conventional cotton on all three rows (CVT-CVT-CVT). Treatments were arranged in a RCB design and replicated four times. A non-planted border row was used to avoid larval movement among treatments. General agronomic and IPM practices for cotton production followed current Louisiana Cooperative Extension Service recommendations.

Tobacco budworm larvae were collected from velvetleaf, *Abutilon theophasti* (Medic.) placed in a plastic bag (15.24 X 7.62 X 38.1 cm) and transported to the laboratory. Larvae were placed individually into 29.6-ml plastic cups containing meridic diet with matching lids. The colony was 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. Pupae were removed from the plastic containers and stored in a 3.79-liter cylindrical cardboard container containing 3 cm of vermiculite. The top of the container was covered with a single later of cotton gauze as a substrate for oviposition. After adult eclosion the moths were fed a 10% sugar-water solution. Gauze sheets, containing eggs, were harvested daily and sealed in plastic bags. Neonates were placed on diet until larvae developed to fourth instars. The diet containing the larvae and the pupal containers were keep at ambient temperature.

Microplots (3 rows x 3.33 ft.) were established in each field plot prior to infestation. Plants were removed on each end of the microplots (3 ft.) to restrict larval movement to plants within plots. A first-position white flower (flowers located at the first fruiting node of a sympodial branch) was infested with a single fourth instar larva. One white flower per plant on each of five plants was infested in each plot. Yellow "snap-on" tags (A. M. Leonard, Piqua, OH), labeled with the date of infestation was attached to a sympodial branch between the main stem and the infested flower pedicel. Additional white flowers were infested on the same row in each plot for five consecutive days.

Plots were evaluated using a shake sheet (3 ft. X 3 ft.). Tobacco budworm damaged and infested squares and bolls were recorded at 7, 14, and 21 days after first infestation date. The larvae recovered from plants were placed back in a first position white flower of plants on the center row. Seedcotton yield was recorded in each plot by hand harvesting each row. The plots were harvested on 10 Oct. Only the results from the center row are reported. Data for larval injury to fruiting forms, infested squares and bolls and seedcotton yield were analyzed with ANOVA and means separated with DMRT.

Heliothine Adult Migration in a Bollgard System

Wire cone traps (Hartstack et al. 1979) baited with artificial sex pheromone lures (Hendricks et al. 1987) were used to collect bollworm and tobacco budworm moths. Traps were placed on the border of the refuge (DeltaPearl) and Bollgard (STV 5599B) fields at predetermined sites beginning in the center of the refuge and continuing in a westerly direction at one-half mile intervals for a distance of two miles. The sample sites are referred to as site 1 being in the center of the refuge and sites 2 through 5 being on the border of the Bollgard field. One trap for each species was placed at each site and the paired traps were 25 feet apart. Collection canisters were sampled weekly. Traps were rebaited biweekly with Hercon Luretape (Hercon Environmental Corp., Emigsville, PA). Trapping was initiated on 25 Jun and ended 10 Oct. The refuge was planted with the 80:20 (Bollgard:Refuge) sprayed option.

Results and Discussion

Microplot Study

Based on seasonal mean (three samples) data showed, Bollgard 2 had significantly fewer damaged and infested squares and bolls when compared to the conventional plot (Fig. 1). The BG2-BG2-BG2 unit had significantly fewer damaged squares and infested bolls compared to the CVT-BG2-CVT. CVT-BG2-CVT units had significantly fewer damaged bolls compared to BG2-BG2-BG2 units. There were no significant yield differences between Bollgard 2 plots. Yield was significantly higher in both Bollgard 2 treatments compared to plots planted with conventional cotton (Fig. 1).

Plots planted with Bollgard 2 significantly reduced the feeding ability and survival of tobacco budworm larvae compared to that in the conventional cotton. Wilson et al. (1980) reported that smaller larvae are found on upper sympodial branches and larger larvae move down throughout the plant canopy of conventional cotton. Ramalho et al. (1984) and Farrar and Bradley (1985) reported that vertical distribution of tobacco budworm larvae was dependant on the developmental stage of the crop and growth stage of the larvae. Farrar and Bradley (1985) also reported that tobacco budworm moves within the plant canopy in search of fruiting structures.

There were more larvae recovered from conventional cultivars compared to Bollgard 2 cultivars in the present study. The BG2-BG2-BG2 and CVT-BG2-CVT units had significantly fewer infested squares and bolls compared to that in the CVT-CVT-CVT plots. Yields were significantly higher in the BG2- BG2-BG2 and CVT-BG2-CVT units compared to the CVT-CVT-CVT unit. In the Bollgard 2 plots, larvae were able to survive on the Bollgard 2 and damage fruiting structures.

Heliothine Adult Migration in a Bollgard System

During the test period, the total number of bollworm moths collected among sample sites ranged from 2199 to 3821, and averaged 3422 moths per site (Fig. 2). In July, the greatest number of moths (1306) were collected at site 5 and declined to 978 at site 1. In August, number of moths ranged 928 to 196 with the greatest number collected at site 5 and the lowest at site 1. Moth numbers ranged from 1330 (site 4) to 663 (site 1) in September. In October, the greatest number of moths (875) was collected at site 2 and lowest number (345) at site 1.

The total number of tobacco budworm moths ranged from 818 to 136 among test sites (Fig. 2). The average number collected for each site was 426 moths. In July, the greatest number of moths (126) were collected at site 1 and declined to 44 at site 5. In August, number of tobacco budworm moths ranged from 192 (site 2) to 22 (site 5). Moth numbers ranged from 350 (Site 2) to 42 (Site 4) in September. In October, the greatest number of moths were collected at site 3 (133) and site 5 (0).

Bollworms were the predominate species collected during the test period. A substantial acreage of field corn was located within a radius of two miles and could have influenced bollworm trap captures. Total bollworm numbers increased dramatically at site 2 and at sample sites adjacent to Bollgard cotton compared to number collected at the sample site in the refuge. Insecticide applications probably reduced the number of bollworms emerging in the refuge. The highest number of tobacco budworms was collected at the sample site in the refuge and one-half mile from the refuge. Beyond sites 1 and 2, the number of TBW continually declined.

References

Bacheler, J. S., and D. W. Mott. 1997. Efficacy of grower-managed Bt cotton in North Carolina, pp. 858-861. *In* P. Dugger and D. A. Richter [eds.], Proc. 1997 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Benedict, J. H., E. S. Sachs, D. W. Altman, D. R. Ring, T. B. Stone, and S. R. Sims. 1993. Impact of δ-endotoxin-producing transgenic cotton on insect-plant interactions with *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae). Environ. Entomol. 22: 1-9.

Gore, J., B. R. Leonard, and J. J. Adamczyk. 2001. Bollworm (Lepidoptera: Noctuidae) survival on 'Bollgard' and 'Bollgard 2' cotton flower bud and floral components. J. Econ. Entomol. 94: 1445-1451.

Gore, J., B. R. Leonard, and R. H. Gable. 2003. Distribution of Bollworm, *Helicoverpa zea*(Boddie), injured reproductive structures on genetically engineered *Bacillus thuringiensis* var. *kurstaki* Berliner cotton. J. Econ. Entomol. 96: 699-705.

Farrar, R. R. and J. R. Bradley, Jr. 1985. Within plant distribution of *Heliothis* spp. (Lepidoptera: Noctuidae) eggs and larvae on cotton in North Carolina. Environ. Entomol. 14:205-209

Gould, F., and B. Tabashnik. 1998. Bt-cotton resistance management, pp. 65-105. *In* M. Mellon and J. Rissler [eds.], Now or Never: Serious New Plans to Save a Natural Pest Control. Union of Concerned Scientists, Cambridge, MA.

Greenplate, J. T., S. R. Penn, Z. Shappley, M Oppenhuizen, J. Mann, B. Reich, and J. Osborn. 2000. Bollgard 2 efficacy: quantification of total lepidopteran activity in a 2-gene product, pp. 1041-1043. *In* J. R. Ruberson [ed.], Handbook of Pest Management. Marcel Dekker, Inc. New York, NY.

Hartstack, A. W., D. E. Hendricks, J. D. Lopez, E. A. Stadelbacher, J. R. Philips, and J. A. Witz. 1979. Adult sampling, pp. 105-131. *In* Economic Thresholds and Sampling of Heliothis Species on Cotton, Corn, Soybeans and Other Host Plants. South. Coop. Series Bull. 213.

Hendricks, D. E., T. N. Shaver, and J. L. Goodenough. 1987. Development of bioassay of molded polyvinyl chloride substrates for dispensing tobacco budworm (Lepidoptera: Noctuidae) sex pheromone bait formulations. Envirn. Entomol. 16:605-613.

Leonard, B. R., H. Fife, K. Torrey, J. B. Graves, and J. Holloway. 1997. *Helicoverpa/Heliothis* management in NuCOTN and conventional cotton cultivars in Louisiana, pp. 863-867. *In* P. Dugger, and D. A. Richter [eds.], Proc. 1997 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Luttrell , R. G., L. Wan, and K. Knighten. 1999. Variation in susceptibility of noctuid (Lepidoptera) larvae attacking cotton and soybean to purified endotoxin proteins and commercial formulations of bacillus thuringiensis. J. Econ. Entomol. 92: 21-32.

Mallet, J. and P. Porter. 1992. Preventing insect adaptation to insect-resistant crops: Are seed mixtures or refugia the best strategy? Proc. Roy. Soc. of Lond. 250: 165-169.

Matten S. R. 2001. EPA update on insect resistance management for Bollgard™ cotton, pp. 840-844. *In* P. Dugger and D. A. Richter [eds.], Proc. 2001 Beltwide Cotton Conf. National Cotton Council, Memphis, TN.

Parker, C. D., Jr. 1997. Effects of mixtures of delta-endotoxin-producing transgenic cotton and 'Coker 312' cotton on adult oviposition and larval behavior of *Heliothis virescens* (Lepidoptera: Noctuidae). M. S. thesis, Mississippi State University, Starkville, MS.

Perlak, F. J., R. W. Deaton, T. A. Armstrong, R. L. Fuchs, S. R. Sims, J. T. Greenplate, and D. A. Fischoff. 1990. Insect resistant cotton plants. Biotechnology 8: 839-943.

Ramalho, F. S., J. C. McCarty, and J. N. Jenkins, and W. L. Parrott. 1984. Distribution of tobacco budworm (Lepidoptera: Noctuidae) larvae within cotton plants. J. Econ. Entomol. 77:591-594.

Stewart, S. D., J. J. Adamczyk, Jr., K. S. Knighten, and F. M. Davis. 2001. Impact of Bt cottons expressing one or two insecticidal proteins of *Bacillus thuringiensis* Berliner on growth and survival of Noctuid (Lepidoptera) larvae. J. Econ. Entomol. 94: 752-760.

Voth, R. D., J. T. Greenplate, J. E. Mann, and J. W. Mullins. 2001. Bollgard 2 cotton technical review, p. 883. *In* P. Dugger and D. A. Richter [eds.], Proceedings Beltwide Cotton Conference. National Cotton Council, Memphis, TN.

Wilson, L. T., A. P. Gutierrez, and T. F. Leigh. 1980. Within-plant distribution of the immatures of *Heliothis zea* (Boddie) on cotton. Hilgardia 48(2):12-23.

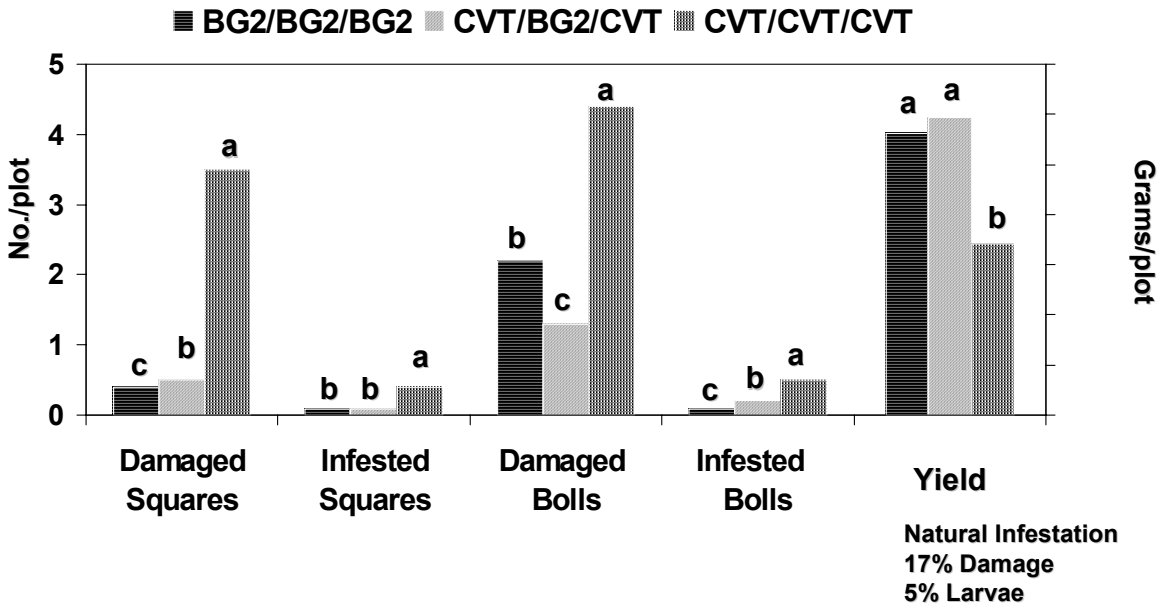

Figure 1. These data represent damaged and infested squares and bolls and yield per plot (Bollgard 2 = BG2; Conventional = CVT).

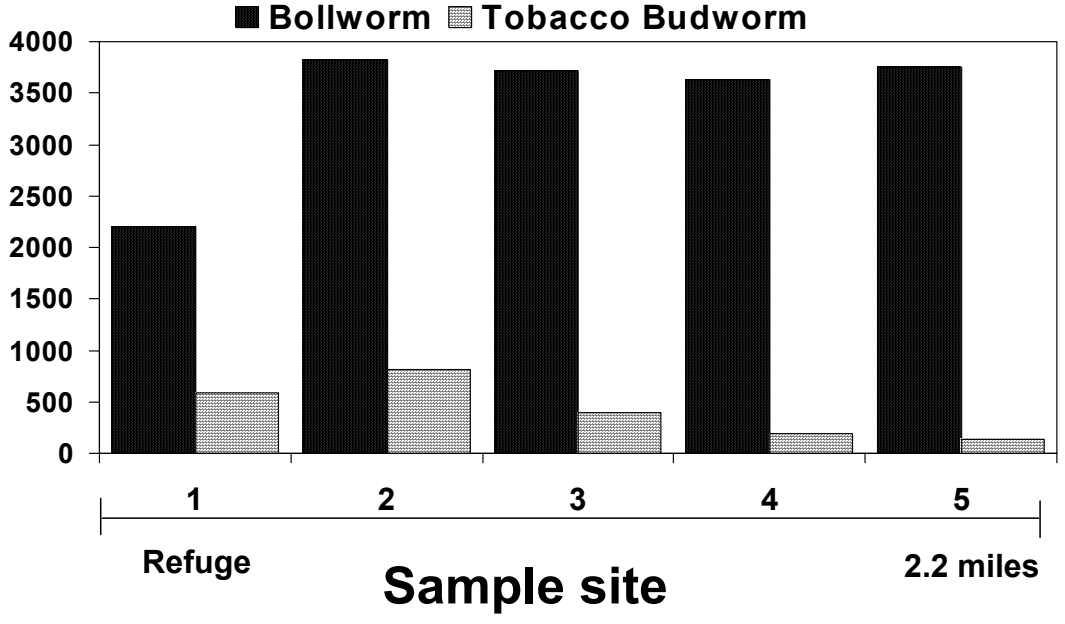

Figure 2. Cumulative number of bollworm and tobacco budworm collected at each sample site.