EFFECTS OF LARGE SCALE BT COTTON PRODUCTION ON TOBACCO BUDWORM POPULATIONS Wade Worley, Frank Mitchener, Tucker Miller III, R. G. Luttrell, and J. C. Schneider Consultant and Grower, Mitchener Farms; Consultant, Miller Entomological Services; and Professors, Mississippi State University, respectively

Abstract

Pheromone trap captures and egg densities of tobacco budworm were monitored in large blocks of Bt and non-Bt cotton in Tallahatachie County, MS, to examine the effects of large scale Bt cotton production on tobacco budworm populations. Data from the unreplicated observations indicated that tobacco budworm populations were reduced 40 to 60% during July and August in the center of a management unit approximately 0.6 miles in radius. Bt cotton provided effective control of tobacco budworm season long, even when populations reached densities 10 fold those typically triggering insecticide applications. Laboratory colonies established from males collected in the Bt cotton did not exhibit measurable levels of resistance to The amount of population reduction Bt endotoxin. observed in the Bt cotton was much more than that expected for the size of the area planted to Bt cotton using previously obtained estimates of tobacco budworm movement for May and June. This suggests that tobacco budworm movement during later periods of the growing season may be less than that previously estimated for May and June when cotton is actively flowering. Given the importance of population level effects on resistance management and area-wide manage-ment strategies, more detailed studies of within season movement of tobacco budworm are needed.

Introduction

Transgenic cotton expressing endotoxin protein of *Bacillus thuringiensis* (Bt cotton) has been the focal point of this conference and much research over the past 5 years. Research and development activities have evolved from the creation of transgenic-insecticidal plants in 1987 to the anticipated commer-cialization of cottons expressing the Bollgard gene in 1996. Essential steps in this process have been the intial field testing of Bt cotton in small field plots in 1989, expanded field plot studies in 5 acre plots in 1992, on-farm strip tests by seed companies in 1994, and production level tests in grower fields in 1995. Different researchable questions have been addressed at each step in the process. The initial small plot studies were conducted to confirm activity of the insecticidal plants against target

pests (Jenkins et al. 1993). The expanded 5 acre plots were designed to study the effect of Bt cotton on the total arthropod complex on small scale (Luttrell et al. 1995). On-farm field tests during 1995 were conducted to estimate the total insecticide inputs required for Bt cotton as compared to traditional inputs on non-Bt cotton (Reed et al. 1996).

While the efficacy of Bt cotton against target pests is relatively well understood, the impact of large, contiguous areas of Bt cotton on associated arthropods is not well understood. The research arena available has been too small to accurately study these relationships. As Bt cottons are commer-cialized and larger areas are planted to the insecticidal plants, more opportunites will be available to study these issues. However, some of the potential impacts of Bt cotton on population dynamics of major pests, like the tobacco budworm (Heliothis virescens), are of immediate interest to cotton growers. Growers are interested in estimating the population suppression effects of Bt cotton and the potential "area-wide" or "farm-wide" benefits associated with growing large areas of these highly effective insecticidal plants. Area-wide management of tobacco budworm has been a long time goal of growers in the Mississippi delta. A diversity of different control methods including destruction of wild host plants on roadsides and field borders, mass release of parasitic wasps, mass release of the Heliothis virescens X Heliothis subflexa backcross which produces sterile male progeny, and areawide spraying of insect viruses have been studied and developed to varying degrees. A common factor in the potential efficacy of these control measures is the size of the area managed (Schneider et al. 1989). Over time, pest populations will reinfest and fill-in areas where pest populations have been reduced. Thus, the overall benefit of reducing pest populations on an area-wide basis is greatly influenced by mobility of the pest species and the size of the area managed. With tobacco budworm, these influences have been studied in diffusion-based models (Turchin and Thoeny 1992) using parameter estimates from data collected to estimate overwinter-ing densities of tobacco budworm and suppression of tobacco budworm following releases of the Heliothis virescens X Heliothis subflexa backcross for the May to June generation. Model results indicate that the tobacco budworm is highly mobile and that management units should be larger than individual farms. Fifty percent of the insects were estimated to have moved distances greater than 6 to 15 miles from the release site. This amount of movement would effectively "wash out" or overwhelm any local variation in population densities at the farm level. Data on movement of the tobacco budworm during the cotton growing season are not available, but large areas of flowering cotton may provide sufficient host plants to reduce movement and increase the effectiveness of population management efforts on a farmlevel scale.

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During 1995, we had an opportunity to examine the effects of Bt cotton on population suppression of tobacco budworm at such a scale. Densities of tobacco budworm moths and eggs were monitored in a 2500 acre block of Bt cotton and compared to those monitored in adjacent blocks of non-Bt cotton. Because of the high insecticidal activity of Bt cotton (Mascarenhas 1994) and the low probability of emergence of tobacco budworm moths from Bt cotton, these observations allowed us to project the effects of local population suppression on recolonization of the Bt cotton during July and August. Moths captured in the pheromone traps were also used to establish laboratory colonies which were tested for Bt resistance. Moths captured in the center of large areas of Bt cotton could be more resistant than those captured in non-Bt cotton because of intense selection for resistance. Although the observations were not geographically replicated and included only one year of data, they represent some of the first data on the impact of large acreages of Bt cotton on tobacco budworm populations. Results should be interpreted with caution because of the limited temporal and geographic characteristics of the data base.

Materials and Methods

All observations were made during 1995 on Mr. Frank Mitchener's farm near Sumner, MS. During mid-June, wire cone traps were placed at 3 locations near the center of a 2500 acre block of Deltapine NuCotn 33 (Bt cotton). The 3 traps were located 0.3, 0.5, and 0.8 miles from the border of the Bt cotton. Three additional traps were placed at similar distances into an adjacent 500 acre block Deltapine 5409 (non-Bt cotton). This 500 acre block of Deltapine 5409 was bordered by large acreages of other non-Bt cotton varieties on adjacent farms. The traps were initially baited on June 20 and were monitored every 4 days until August 31. The pheromone was replaced in all traps every 14 days. Densities of eggs oviposited on the Bt and non-Bt cotton plots were obtained from routine scouting data collected by the agricultural consultants responsible for the acreage involved. Fields were typically scouted twice each week.

On 5 separate dates during the observation period, tobacco budworm male moths were collected from the pheromone traps and transported to research laboratories at Mississippi State University (Table 1). These males were mated with virgin females from a laboratory colony considered to be susceptible to Bt endotoxin. Because resistance to Bt endotoxin is expected to be inherited as a single recessive gene, the F₁ progeny were crossed and the F₂ to F₄ generations were exposed as neonates to Bt endotoxin (HD-73 CryIa(c)) in a diet incorporation assay. The dose used was a LC-90 determined from previous experiments at Mississippi State University (Wan 1995).

Results and Discussion

Based on pheromone trap captures (Table 2 an Figure 1) and scouting data (Table 3 and Figure 2), the Bt and non-Bt cotton were colonized by similar densities of tobacco budworm during June. The relationship between pheromone trap captures and egg densities were similar in both Bt and non-Bt cotton (Figures 3 and 4). Data were not collected for pheromone trap captures prior to June 20, and average trap captures for the late June through early July period may have included some moths that had fed as larvae on cotton. However, trap captures in the Bt cotton were slightly higher than those in the non-Bt cotton during late June and early July. If some of these moths were from larvae that developed on cotton, they probably moved into the Bt cotton from non-Bt cotton because survival of tobacco budworm larvae on Bt cotton is extremely rare (Mascarenhas 1994). Scouting data indicated similar densities of eggs on Bt and non-Bt cotton during early June. Densities during late June and early July (6/15-7/14 in Table 3) were reduced in the Bt cotton as compared to the non-Bt cotton but overall densities were low.

Pheromone trap captures in the Bt cotton were reduced by 49 and 34% of those in the non-Bt cotton for periods of time coinciding with the emergence of adults from the first (7/15-8/14) and second (8/15-8/30) generations of tobacco budworm produced on cotton. Densities of tobacco budworm eggs (Table 3) were also reduced (67 and 64%, respectively) in the Bt cotton as compared to the non-Bt cotton during late June through early July (6/15-7/14 in Table 3) and during late July through early August (7/15-8/14 in Table 3). The late August or third generation of tobacco budworm eggs on cotton (8/15-8/31 in Table 3) were at extremely high densities (more than 10 fold those observed earlier during the growing season). Although these densities were much higher than treatment threshold levels, densities in the Bt cotton were 17% less than those in the non-Bt cotton (Table 3). Previous studies in 5 and 25 acre plots (Mascarehas et al. 1995 and Parker unpublished data) have shown equal densities of eggs in Bt and non-Bt cotton throughout the growing season. Differences observed between Bt and non-Bt cotton in 1995 on Mr. Mitchener's farm suggest that plot size is important and that population suppression effects may be observable on areas the scale of Mr. Mitchner's farm. The previous plots may have been too small. Although population suppression may have been observed, the amount of local suppression of tobacco budworm populations was not sufficient in itself to reduce late season infestation densities below those considered to be damaging in non-Bt cotton. The Bt cotton provided excellent control of tobacco budworm larvae, and the crop did not experience fruit loss to tobacco budworm. Reductions observed in egg densities and pheromone trap captures in the large block of Bt cotton suggest that population suppression can be observed on the scale of a large farm. If these observations are accurate, the amount of movement tobacco budworm exhibits during July and August when cotton is actively fruiting is less than the

amount of movement previously estimated for May to June generations.

To further examine the difference in tobacco budworm movement between those observed for mid- to late-season and those previously estimated for early-season generations, the diffusion model describing early-season movement was run using the average distance from the border of the Bt cotton to the location of traps within that cotton (0.6 mile). The model based on the lowest average distance moved per generation from the previous early-season indicated that the population in the middle of the Bt cotton would be expected to be reduced by less than 3%. This amount of reduction is much less than the 40 to 60% observed. When the model was run using a management area with a radius of 1.2 miles (twice that observed in 1995), the next generation of tobacco budworm was expected to be reduced by less than 6%. The large difference between observed reductions and those expected from previous experiments raises important questions relative to our know-ledge of tobacco budworm movement during the cotton growing season. Estimates based on data collected during May to June may not be suitable for projecting population suppression effects during later periods of the growing season when cotton is an attractive, flowering host plant. The limited observations made during this study need to be further expanded. These preliminary results are suggestive that management decisions made on a single farm can have observable consequences later in the growing season. Cooperation among farmers and deployment of management strategies over larger areas should enhance the effects of area-wide management on tobacco budworm populations, but the size of the area required may be smaller for within season management than for early season management of tobacco budworm populations.

A total of 8075 progeny of the crosses between wild tobacco budworm males collected in the Bt cotton and laboratory susceptible females was assayed for resistance to Bt endotoxin. Larvae were exposed to a LC-90 dose (Wan 1995) in a diet-incorporation assay. Only 16 larvae survived (less than 1%) the 7 day observation period, and none survived long enough to emerge as healthy females (Table 4). This rate of survival was actually less than the 10% expected which suggested that no Bt resistant genes were associated with the males captured in pheromone traps.

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References

Jenkins, J. N., W. L. Parrott, J. C. McCarty, F. E. Callahan, S. A. Berberich, and W. R. Deaton. 1993. Growth and survival of *Heliothis virscens* (Lepidoptera: Noctuidae) on transgenic cotton containing a trucated form of the delta endotoxin gene from *Bacillus thuringiensis*. J. Econ. Entomol. 86:181-185.

Luttrell, R. G., V. J. Mascarenhas, J. C. Schneider, C. D. Parker, and P. D. Bullock. 1995. Effect of transgenic cotton expressing endotoxin protein on arthropod populations in Mississippi cotton. pp. 760-763. In Proc. Beltwide Cotton Prod. Res. Conf., National Cotton Council, Memphis, TN.

Mascarenhas, V. J. 1994. Direct and indirect effects of transgenic cotton expressing the delta-endotoxin of *Bacillus thuringiensis* on survival of tobacco budworm (*Heliothis virescens*) and bollworm (*Helicoverpa zea*). M.S. Thesis, Mississippi State University, 102 pp.

Reed, J. T., C. D. Parker, R. G. Luttrell, F. A. Harris, and S. Stewart. 1996. The MAFES cotton insect pest management project: overview and first year results. In Proc. Beltwide Cotton Prod. Res. Conf., National Cotton Council, Memphis, TN.

Schneider, J. C., R. T. Roush, W. F. Kitten, and M. L. Laster. 1989. Movement of *Heliothis virescens* (Lepidoptera: Noctuidae) in Mississippi in the Spring: Implications for area-wide management. Environ. Entomol. 18:438-446.

Turchin, P. and W. T. Thoeny. 1993. Quantifying dispersal of southern pine beetles with mark-recapture experiments and a diffusion model. Ecolog. Appl. 3:187-198.

Wan, Li. 1995. Susceptibility and avoidance behavior to *Bacillus thurin-giensis* endotoxin in noctuid larvae attacking cotton and soybean. M.S. Thesis, Mississippi State University, 137 pp.

Table 1. Laboratory colonies established from moths captured in pheromone traps located in Bt cotton.

		Number	Number
	Date	of Wild	of Laboratory
Colony	Collected	Males	Females
RBT95-1	7/21	1	10
RBT95-2	7/26	12	54
RBT95-3	7/31	34	31
RBT95-5	8/3	45	43
RBT95-6	8/25	16	40

Table 2. Tobacco budworm moths captured in pheromone traps placed in 2500 acre blocks of Bt and non-Bt cotton.

	Moths/Tra	p/Night		
Percent				
Dates	Bt	non-BT	Reduction	
6/15 - 7/14	4.4	2.6	0.0	
7/15 - 8/14	4.7	9.2	48.9	
8/15 - 8/30	7.0	10.6	34.0	

Table 3. Tobacco budworm eggs found in 2500 acre blocks of Bt and non-Bt cotton.

Average Number of Eggs				
		Per 100 Terminals	Percent	
Dates	Bt	non-Bt	Reduction	
6/1 - 6/15	6.5	7.0	7.0	
6/15 - 7/14	0.6	1.7	66.9	
7/15 - 8/14	4.4	12.4	64.4	
8/15 - 8/31	62.5	75.0	16.7	

Table 4. Results of laboratory monitoring of tobacco budworm progeny for resistance to Bt endotoxin.

	Generation	Number	Number	Percent
Colony	Tested	Tested	Surviving	Survival
RBT95-1	F4	972	3	0.3
RBT95-2	F2	888	2	0.2
RBT95-2	F4	240	1	0.4
RBT95-3	F2	288	1	0.4
RBT95-3	F3	720	0	0.0
RBT95-3	F4	600	2	0.3
RBT95-5	F3	456	0	0.0
RBT95-5	F5	72	0	0.0
RBT95-6	F2	1104	0	0.0
RBT95-6	F3	2736	7	0.3
Total		8076	16	0.2



Figure 1. Pheromone trap captures in Bt and non-Bt cotton



Figure 2. Densities of tobacco budworm eggs in Bt and non-Bt cotton.



Figure 3. Relationship between egg densities and pheromone trap captures in Bt cotton.



Figure 4. Relationship between egg densities and pheromone trap captures in non-Bt cotton.