2003 BELTWIDE COTTON CONFERENCES, NASHVILLE, TN – JANUARY 6-10

QUANTIFICATION OF *HELICOVERPA ZEA* POPULATIONS IN EASTERN NORTH CAROLINA CROP ENVIRONMENTS: IMPLICATIONS FOR *B. T.* RESISTANCE MANAGEMENT

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Abstract

Bollworm, *Helicoverpa zea* (Boddie), production from various crop hosts was measured in 2002 by weekly larval monitoring in commercial crop fields and side-by side plantings to determine if temporal and spatial production of *H. zea* from various crop hosts are effective for *B. t.* resistance management. Pheromone trap catches demonstrated that *H. zea* production in localized areas had little impact on the number of *H. zea* moths in the local environment, which suggests a high level of movement by *H. zea* adults. Results from larval monitoring in commercial fields and a small plot trial demonstrated the potential for alternate crop hosts to produce much higher numbers of *H. zea* larvae and adults compared to Bollgard® cottons. Bollgard cottons produced the fewest number of larvae in six of six weeks for commercial field monitoring and four of five weeks for small plot trial monitoring. Therefore, results show that temporal and spatial production of *H. zea* by various crop hosts favor *B. t.* resistance management and that alternate crop hosts can effectively supplement the 5% unsprayed cotton refuge for Bollgard and Bollgard II® cottons.

Introduction

The U. S. Environmental Protection Agency granted a five-year extension for the general Bollgard® (Monsanto Co., St. Louis, MO) registration late in 2001. However, the 5% unsprayed refuge option will remain in effect only until the end of 2004, at which time it will be reviewed by EPA to determine whether or not this specific option will be continued. At that time, the EPA may require changes in refuge size, structure, and deployment as a means of achieving the goal of producing 500 susceptible adult bollworms, *Helicoverpa zea* (Boddie), in the refuge for each adult carrying a resistance allele (Matten 2001). Jackson et al. (2001, 2002) reported production of bollworm adults from conventional cotton to be 13-33X that produced in Bollgard and 64-96X that produced in Bollgard II in North Carolina field studies that were not sprayed with insecticide. These estimates are somewhat conservative since a portion of the survivors on Bollgard and Bollgard II cottons may not have carried resistance alleles. However, previous laboratory studies (Burd et al. 2000, 2001) have demonstrated the increased potential for these survivors to possess alleles conferring resistance to Bollgard cottons, which suggests the probability that survivors carry resistance alleles. In addition, the adult production estimates presented above represent a 1:1 ratio of conventional cotton to transgenic cotton. In a refuge deployment option of 95:5 (Bollgard: conventional cotton), production of bollworm adults from conventional cotton would be 1.76X that produced in Bollgard cottons based on production estimates. These results suggest that *H. zea* moth production in refuge cotton has not approached the numbers desired for resistance management. Additionally, our studies have shown no trend toward resistance development suggesting that moth production on alternate hosts has been more substantial than originally thought and/or there is an unknown fitness cost associated with *B. t.* resistance development in bollworm.

The agroecosystems in North Carolina are characterized by a high degree of crop host diversity. Diversity in this region is impacted by small field sizes relative to other production regions in the U. S. cotton belt. For example, the average cotton field in the area has been estimated at 15 acres by Boll Weevil Eradication personnel. Intercrop movement of *H. zea* adults is enhanced by the spatial heterogeneity imposed by small field sizes; thus, the probability of matings between moths produced on *B. t.* and refuge crops is high in this region.

The objectives of the study reported herein were 1) to quantify temporal and spatial *H. zea* production from crop hosts (corn, cotton, peanut, and soybean) in targeted agroecosystems representative of North Carolina agriculture and 2) to determine temporal *H. zea* production from side-by-side strip plantings of crop hosts (corn, cotton, peanut, and soybean).

Materials and Methods

Production of *H. zea* from various crop hosts was measured using two methods: weekly monitoring of commercial crop fields and side-by side plantings for larval and adult production. Four cotton-producing counties were selected for monitoring of *H. zea* production in commercial crops. These four counties (Chowan, Edgecombe, Halifax, and Washington) were selected because of variation in production of Bollgard cottons and alternate host crops among counties. H. zea larval and adult production was monitored in five areas within each county that consisted of an interface of a Bollgard cotton field with another crop host field (Bollgard cotton, conventional cotton, corn, peanut, or soybean). Two modified Harstack pheromone traps were placed along each crop interface with a minimum distance of 100 yards between traps and baited with Luretape® (Hercon

Environmental Co., Emigsville, PA). Adult *H. zea* were collected each week from pheromone traps and counted to compare moth production at each interface. *H. zea* larval sampling was also conducted on a weekly basis at each crop interface by sampling 100 row ft. within each crop at each interface. Each crop field associated with pheromone traps were sampled for large (L4-L5) larvae in order to estimate the proportion of moths produced by each crop at each interface and to determine whether moths captured in pheromone traps were produced at a particular interface. Larval sampling was initiated at the on-set of the *H. zea* moth flight in late July and terminated in late September when all crops had reached maturity and no *H. zea* larvae were found.

Side-by-side strip plantings of the various crop hosts were planted in a randomized complete block design with four replicates at the Tidewater Research Station near Plymouth, NC. Plots were 20 rows wide by 60 ft. in length with a row width of 38 in. Field corn was planted on 24 April, peanut on 6 May, Bollgard and conventional cottons on 14 May, and soybean on 28 May. Crop production practices such as fertilization, irrigation, and weed control were provided as recommended by the North Carolina Cooperative Extension Service. Crops were monitored weekly for numbers of large *H. zea* larvae (L4-L5) by sampling 10 row ft. within each plot. A representative subsample of larvae were collected and taken to the laboratory for rearing. Large larvae were reared on artificial diet in the laboratory until the adult stage. Larval, pupal and adult production were determined for each crop host and were transformed to a per acre basis prior to analysis.

All data were $\log_{10}$ transformed before being subjected to ANOVA using PROC GLM (SAS Institute 1990). Means for each treatment were separated ($P \leq 0.05$) using Fisher's Protected Least Significant Difference test.

Results

Extremely high populations of bollworm were found throughout eastern North Carolina in 2002, as indicated by pheromone trap collections and larval sampling in commercial crop fields (Tables 1 and 2). Mean numbers of bollworm moths collected per trap set per week differed among crop interfaces in only one week from 24 July to 25 September (Table 1). Pheromone trap catches during the week of 27 August showed that the Bollgard/soybean crop interface produced more moths than the interfaces of Bollgard cotton and conventional cotton, field corn, and peanut. The numbers of moths collected from the Bollgard/Bollgard interface was not statistically different from that at the Bollgard/soybean interface. Moth catches at the Bollgard/Bollgard interface were only statistically higher than that at the Bollgard/peanut interface.

H. zea larval numbers in commercial crop fields at each interface varied greatly across sample dates (Table 2). Field corn was the only crop in which large *H. zea* larvae were found on 29 July. However, all crops with the exception of Bollgard cotton were producing similar levels of large *H. zea* larvae by 6 August. On 13 August, *H. zea* larval production did not differ among crop hosts. By 21 August, peanut and soybean produced numbers of *H. zea* larvae similar to that of field corn; however, larval production in these two crop hosts was significantly higher than that of both Bollgard and conventional cottons. Soybean and peanut again produced similar numbers of large *H. zea* larvae on 28 August, but only soybean produced significantly higher numbers of larvae as compared to field corn, Bollgard cotton, and conventional cotton. By 4 September, soybean produced significantly higher numbers of *H. zea* larvae as compared to other crop hosts.

In side-by-side crop plantings, field corn was the only crop that produced large *H. zea* larvae, 13,756 per acre, on the initial sample date of 31 July (Table 3). By 8 August, all crop hosts of *H. zea* produced similar levels of large larvae with the exception of Bollgard cotton. Larval numbers ranged from 344 per acre in Bollgard cotton to 14,100 per acre in conventional cotton. Soybean, conventional cotton, and field corn produced similar numbers of *H. zea* larvae (28,888, 19,258, and 6,190 larvae per acre, respectively), all of which produced significantly more large larvae than Bollgard cotton (688 larvae per acre) on 14 August. Numbers of *H. zea* larvae in soybean were also significantly higher than the 1,720 larvae per acre produced in peanut on this date. On 21 August, similar numbers of *H. zea* larvae were produced by soybean, field corn, conventional cotton, and Bollgard cotton; each of these crop hosts, with the exception of Bollgard cotton, produced significantly higher numbers of *H. zea* larvae compared to peanut. On the last sample date, no significant differences in larval production were evident among crop hosts of *H. zea*. Numbers of *H. zea* larvae per acre ranged from 0 in Bollgard cotton, field corn, and peanut to 688 in soybean on 30 August.

Production of *H. zea* pupae followed the same trends as larval production on all dates except for 21 August in side-by side crop plantings (Table 4). At this time, there were no differences among crops with respect to production of *H. zea* larvae; however, pupal production was similar among all crops except for peanut, which produced the fewest numbers of *H. zea* pupae during this period. Adult production also followed the same trends as larval and pupal production until 14 August (Table 5). On this date, production of *H. zea* pupae was similar among peanut and Bollgard cotton; however, adult production from Bollgard cotton was significantly lower than that of peanut on this date. Production patterns of *H. zea* adults also differed from that of pupal production on 21 August. At this time, pupal production was similar among all crop hosts except peanut, which had the lowest production estimates. Adult production, however, was significantly higher in soybean as compared to Bollgard cotton and peanut on 21 August. Production of *H. zea* adults for conventional cotton and field corn was similar to both that of soybean and Bollgard cotton. *H. zea* adult production patterns were again similar to that of larval and pupal trends on the last sample date.

Discussion

The similarities among pheromone trap catches in nine of ten sampling weeks suggest that bollworm production from crops within a particular localized area has little impact on the spatial occurrence of *H. zea* moths in the local environment. Even though pheromone trap catches differed statistically during the week of 27 August, it is unlikely that the difference was a function of *H. zea* moth production from the particular crop interfaces, based on larval production estimates at these interfaces during the weeks prior to 27 August. This suggests that movement of bollworm moths into and out of localized areas is much more significant than anticipated. Therefore, due to the spatial diversity of crop hosts coupled with small field sizes in North Carolina, the importance of the cotton refuge for Bollgard cottons declines due to the production of *H. zea* from alternate crop hosts and the substantiation of increased adult movement.

H. zea larval production in the small plot trial differed somewhat from that of commercial fields primarily because the small plot trial was not oversprayed with insecticides toxic to bollworm. Therefore, larval production, on average, was higher in the small plot trial. However, results from this trial are useful when comparing *H. zea* production from unsprayed crop hosts, which are typical during years of low to moderate bollworm infestations in North Carolina. Field corn was the major producer of *H. zea* larvae early in the season before crops such as conventional cotton, soybean, and peanut increased bollworm production; however, field corn was a major producer until the last week of August, suggesting that field corn may serve as a refuge for *H. zea* much later in the growing season than originally thought. Soybean and conventional cotton became the major crop sources of bollworm production beginning in mid-August and lasting throughout the testing period. These results demonstrate that alternate host crops are producing susceptible *H. zea* larvae throughout the entire period in which *H. zea* larvae would be produced from *B. t.* crops

Pupal and adult production followed trends similar to that of larval production. Soybean, field corn, and conventional cotton demonstrated the potential to produce much higher numbers of *H. zea* adults compared to that of Bollgard cottons. Although peanut did produce numbers of *H. zea* adults similar to that of Bollgard cotton in the small plot trial, peanut was shown to out-produce Bollgard cottons in commercial situations. These results suggest that any of the crop hosts tested has the ability to produce a significant quantity of susceptible *H. zea* moths to supplement those produced by the 5% unsprayed cotton refuge for *H. zea B. t.* resistance management.

The results provided herein demonstrate the ability of crop hosts such as field corn, soybean, and peanut to supplement the 5% unsprayed conventional cotton refuge both temporally and spatially for effective *B. t.* resistance management of bollworm.

Acknowledgments

The authors express appreciation to Monsanto Agric. Co. for providing project funding. Special thanks are given to Wayne Modlin, Phil Threatt, Margery Ambrose, Ryan Kurtz, and Maria Marcus for technical assistance.

Literature Cited

Burd, A. D., J. R. Bradley, Jr., J. W. Van Duyn, and F. Gould. 2000. Resistance of bollworm, *Helicoverpa zea*, to CryIA(c) toxin. Proc. Beltwide Cotton Conf. 923-926.

Burd, A. D., J. R. Bradley, Jr., J. W. Van Duyn, and F. Gould. 2001. Estimated frequency of non-recessive *B.t.* resistance genes in bollworm, *Helicoverpa zea*. Proc. Beltwide Cotton Conf. 820-822.

Jackson, R. E., J. R. Bradley, Jr., J. W. Van Duyn, and A. D. Burd. 2002. Estimated production of *Helicoverpa zea* adults from Bollgard and Bollgard II cottons and implications for resistance management. Proc. Beltwide Cotton Conf. 815-818.

Jackson, R. E., J. R. Bradley, Jr., J. W. Van Duyn, and A. D. Burd. 2001. Efficacy of Bollgard and Bollgard II cottons against bollworm, *Helicoverpa zea* (Boddie), in field and greenhouse studies. Proc. Beltwide Cotton Conf. 815-818.

Matten, S. R. 2001. EPA update on insect resistance management for Bollgard cotton. Proc. Beltwide Cotton Conf. 840-844.

SAS Institute. 1990. SAS/STAT User's Guide, Vol. 2. SAS Institute, Cary, NC, 795 pp.

Table 1. Mean (SE) numbers of *Helicoverpa zea* adults per two-trap set week at various crop host interfaces averaged across four counties on ten sample dates in North Carolina, 2002.

	24-July	**1-August**	**8-August**	**15-August**	**21-August**
Bollgard cotton	316 a	1,169 a	666 a	326 a	726 a
	(185)	(483)	(184)	(82)	(81)
Conventional cotton	389 a	1,093 a	633 a	312 a	562 a
	(196)	(440)	(258)	(164)	(234)
Field corn	260 a	1,537 a	750 a	419 a	677 a
	(57)	(344)	(137)	(132)	(193)
Peanut	247 a	1,392 a	875 a	542 a	681 a
	(151)	(564)	(386)	(264)	(270)
Soybean	315 a	1,558 a	865 a	512 a	1,007 a
	(147)	(610)	(326)	(243)	(193)
	27-August	**6-September**	**11-September**	**18-September**	**25-September**
Bollgard cotton	943 ab	764 a	871 a	565 a	587 a
	(75)	(113)	(137)	(158)	(128)
Conventional cotton	641 bc	645 a	574 a	382 a	387 a
	(202)	(164)	(166)	(142)	(68)
Field corn	765 bc	716 a	551 a	387 a	425 a
	(167)	(177)	(77)	(60)	(253)
Peanut	516 c	695 a	755 a	470 a	518 a
	(182)	(170	(122)	(81)	(76)
Soybean	1,214 a	1,015 a	896 a	424 a	339 a
	(307)	(231)	(414)	(82)	(72)

Means within the same column followed by the same letter are not significantly different, Fisher's Protected LSD test ($P \leq 0.05$).

Table 2. Mean (SE) numbers of *Helicoverpa zea* larvae (L4-L5) produced per acre at various crop host interfaces averaged across four counties on six sample dates in North Carolina, 2002.

	29-July	**6-August**	**13-August**	**21-August**	**28-August**	**4-September**
Bollgard cotton	0 b	0 b	0 a	0 b	0 b	0 b
	(0)	(0)	(0)	(0)	(0)	(0)
Conventional cotton	0 b	2,339 a	1,170 a	0 b	0 b	0 b
	(0)	(645)	(642)	(0)	(0)	(0)
Field corn	9,079 a	6,637 a	1,513 a	310 ab	0 b	0 b
	(1,272)	(2,671)	(881)	(198)	(0)	(0)
Peanut	0 b	4,299 a	3,198 a	2,820 a	413 ab	103 b
	(0)	(2,397)	(2,310)	(2,413)	(413)	(103)
Soybean	0 b	3,955 a	2,064 a	1,032 a	2,236 a	2,923 a
	(0)	(1,724)	(695)	(399)	(1,963)	(2,183)

Means within the same column followed by the same letter are not significantly different, Fisher's Protected LSD test ($P \leq 0.05$).

Table 3. Mean (SE) numbers of *Helicoverpa zea* larvae (L4-L5) produced per acre within various crop hosts on five sample dates in North Carolina, Tidewater Research Station, 2002.

	31-July	**8-August**	**14-August**	**21-August**	**30-August**
Bollgard cotton	0 b	344 b	688 b	2,407 ab	0 a
	(0)	(344)	(397)	(344)	(0)
Conventional cotton	0 b	14,100 a	19,258 ab	6,534 a	344 a
	(0)	(4,141)	(973)	(659)	(344)
Field corn	13,756 a	7,566 a	6,190 ab	5,502 a	0 a
	(1486)	(2,138)	(1,191)	(1,256)	(0)
Peanut	0 b	3,095 a	1,720 ab	1,032 b	0 a
	(0)	(344)	(865)	(344)	(0)
Soybean	0 b	3,095 a	28,888 a	12,724 a	688 a
	(0)	(1,418)	(2,025)	(4,103)	(397)

Means within the same column followed by the same letter are not significantly different, Fisher's Protected LSD test ($P \leq 0.05$).

Table 4. Mean (SE) numbers of *Helicoverpa zea* pupae produced per acre within various crop hosts on five sample dates in North Carolina, Tidewater Research Station, 2002.

	31-July	8-August	14-August	21-August	30-August
Bollgard cotton	0 b	344 b	688 c	2,407 a	0 a
	(0)	(344)	(397)	(344)	(0)
Conventional cotton	0 b	13,068 a	17,195 ab	6,190 a	344 a
	(0)	(3,485)	(1,637)	(688)	(344)
Field corn	13,412 a	7,566 a	6,190 ab	5,502 a	0 a
	(1,302)	(2,138)	(1,191)	(1,256)	(0)
Peanut	0 b	3,095 a	1,720 bc	688 b	0 a
	(0)	(344)	(865)	(397)	(0)
Soybean	0 b	2,751 a	25,449 a	11,693 a	688 a
	(0)	(1,256)	(2,063)	(3,991)	(397)

Means within the same column followed by the same letter are not significantly different, Fisher's Protected LSD test ($P \leq 0.05$).

Table 5. Mean (SE) numbers of *Helicoverpa zea* adults produced per acre within various crop hosts on five sample dates in North Carolina, Tidewater Research Station, 2002.

	31-July	8-August	14-August	21-August	30-August
Bollgard cotton	0 b	344 b	344 c	1,376 b	0 a
	(0)	(344)	(344)	(562)	(0)
Conventional cotton	0 b	11,693 a	15,476 a	5,502 ab	344 a
	(0)	(2,543)	(1,175)	(562)	(344)
Field corn	13,068 a	6,878 a	5,502 ab	5,159 ab	0 a
	(1,317)	(1,945)	(973)	(1,032)	(0)
Peanut	0 b	3,095 a	1,720 b	344 c	0 a
	(0)	(344)	(865)	(344)	(0)
Soybean	0 b	2,751 a	22,010 a	10,661 a	344 a
	(0)	(1,256)	(1,256)	(3,433)	(344)

Means within the same column followed by the same letter are not significantly different, Fisher's Protected LSD test ($P \leq 0.05$).