Chapter 4

Diversity of Helicoverpa armigera (Lepidoptera: Noctuidae) Damaged Reproductive Structures on Genetically Modified, (Bt) Cotton plant

Introduction

In India, cotton insect pest management is becoming more complex with the introduction of genetically modified (GM) cotton plants (Bollgard®) varieties. Bollgard® cotton plants produce the insecticidal Cry1Ac protein from Bt (Bacillus thuringiensis) Kurstaki Berliner (Perlak et al. 1990) that is δ endotoxin to larvae from the insect order Lepidoptera (MacIntosh et al. 1990). Helicoverpa virescens (F.); Pectinophora gossypiella (Saunders); and Helicoverpa armigera (Hubner) all are susceptible to Cry1Ac and have been the primary targets of Bollgard® cotton plant. However, under certain situations, the Cry1Ac protein produced by Bollgard® cotton plant has provided less than adequate control of bollworms (Stewart et al. 2001). Several factors contribute to bollworm infestations becoming established in Bollgard® cultivars. Helicoverpa armigera, bollworms are inherently less toxic to the Cry1Ac protein in Bollgard® (Luttrell et al. 1999). Also, Cry1Ac levels vary both temporally (Greenplate 1999) and spatially (Greenplate 1999, Adamczyk et al. 2001). In general, protein expression in bolls is lower than in squares (Adamczyk et al. 2001).

The population of Helicoverpa armigera feeding on older reproductive structures low in the plant canopy during the late season may not be controlled as effectively by Bollgard® cotton plant as populations during the early fruiting period or on younger structures in the upper portions of the plant canopy. The survival of Helicoverpa armigera is higher in white flowers than in other reproductive structures (Gore et al. 2001). Consequently, injury to white flowers is greater than for other plant structures (Gore et al. 2000a). The temporal and spatial variability in protein expression in Bollgard® plants coupled with differences in larval behaviour, results of bollworm populations becoming established low in the plant canopy.

However, no information is currently available characterizing the levels
of injury those populations cause Bollgard® has not provided acceptable levels of bollworm control in all situations. Therefore, a second generation of genetically engineered insect resistant cotton plants is being developed by Monsanto Co. USA. These experimental cotton plant lines express two separate *Bacillus thuringiensis* (Bt) proteins (Cry1Ac and Cry2Ab) to improve efficacy against bollworms, *Helicoverpa armigera* and other lepidopteran pests (Greenplate et al. 2000a).

Cry1Ac protein expression in Bollgard® II is similar to the level of Cry1Ac expressed in Bollgard®. The addition of Cry2Ab in Bollgard® II increases the amount of total insecticidal protein produced by Bollgard® (Greenplate et al. 2000b). Bollgard® II cotton lines have demonstrated significantly better control of *Helicoverpa armigera* and other lepidopteran pests than that observed with Bollgard® cotton (Gore et al. 2001, Stewart et al. 2001).

Before Bollgard® and Bollgard® II cottons can be fully integrated into pest management systems in India, research needs to be conducted to determine if and when insecticide applications should be initiated for bollworm control. Currently, the levels of fruiting form injury caused by *Helicoverpa armigera* after they leave white flowers have not been quantified on Bollgard® cotton plant. Therefore, this study was conducted to determine the level of fruiting form injury from *Helicoverpa armigera* larvae feeding in white flowers of Bollgard® and Bollgard® II cottons.

**Materials and Methods**

Two studies were conducted at the Village Kakani Thasil Sakri of Dhule District of Maharashtra State in field plots during 2009-2010. The first experiment evaluated a commercial Bollgard® variety (cv. Rasi 134, Rasi Seeds (P) Ltd. India) that produces a single *Bacillus thuringiensis* (Bt) protein (Cry1Ac) and its closest conventional (non-transgenic) parental variety (cv. Rasi 2000). In the second experiment, an experimental genetically modified cotton plant that produces two *Bacillus thuringiensis* proteins (Rasi 134 BGII, Cry1Ac+Cry2Ab) was evaluated along with a similar commercial Bollgard®
variety (cv. Rasi 134, Cry1Ac) and conventional parental variety (cv. Rasi 2000). Multiple seeding dates were used in the first study so that plants at the proper stage for infestations would be available over an extended period of time, thereby increasing the overall sample size. Plots (16 rows by 15m) of Rasi 2000 and Rasi 134 were planted during 12th June 2009 to 18th June 2009. Plots (1 row by 20m) of Rasi 2000, Rasi 134, and experimental Bollgard® II (Rasi 134BGII) were planted on 12th June 2009. The *Helicoverpa armigera* larvae were collected from clover, *Trifolium* spp., during late April and from sweet corn, *Zea mays* L., during early June each year. Colonies were maintained in the laboratory for one generation to obtain sufficient numbers of larvae at the proper developmental stage for infestations. The *Helicoverpa armigera* larvae were fed meridic diet in individual 29.5 ml plastic cups. Adults were maintained in 2.79Liter cardboard buckets and fed a 10% sucrose solution. The tops of the buckets were covered with cheesecloth to provide a surface for oviposition. Egg sheets were harvested daily and placed into plastic bags. Upon eclosion, neonates (ca. 50/cup) were fed meridic diet in 236 ml waxed paper cups for 24hr. Larvae were fed diet for 24hr before being placed onto plants to minimize mortality from handling neonates in the field.

**Infestation Procedures**

Cotton plants used in these experiments were at the six to nine main stem nodes above white flower growth stages. Plant growth stages were determined by counting the number of main stem nodes from the uppermost first position white flower to the last unfolded leaf in plant terminals (Bourland et al. 1992). Individual plants were randomly selected within field plots and isolated by removing surrounding plants so that no interplant movement could occur. White flowers at the site of infestation were tagged with a yellow snap on tag.

In the first experiment, two first instar bollworm larvae (24-8hr old) were placed in first position white flowers on single plants from each variety using a small paint brush. Fifty Rasi 2000 (conventional) and Rasi 134 (Bollgard®) plants were infested on each day Infestations were arranged in a randomized
complete block design with d of infestation representing blocks (replicates).

Larvae were placed on separate cohorts of plants on three and five dates during 2009 and 2010, respectively. In the second study, plant availability was limited; therefore, only 10 plants were infested on each day for Rasi 2000, Rasi 134, and the Bollgard® II line. Infestation procedures followed those described for the first experiment. Plants were infested on six different dates for these varieties in 2009.

Plants in both experiments were visually inspected at 3rd day for damage to the fruiting structure at the infested site and for the presence of larvae. Thereafter, entire plants were inspected every second day for cumulative damage to fruiting structures (squares, white flowers, bolls) until larvae were no longer present. In addition to the infested plants, non-infested plants were monitored for natural abscission of fruiting structures.

Data for cumulative numbers of damaged fruiting forms were analysed using repeated measures analysis of variance where damage was recorded from the same experimental units over different rating intervals (Littell et al. 1996). Data for total number of damaged fruiting forms by an individual larva was analysed using analysis of variance (Littell et al. 1996).

**Results**

Bollworms, *Helicoverpa armigera* damaged more fruiting forms on conventional cotton plant than on Bollgard® cotton plant. Effects for cotton type \( (F=7.17; \, df=1, \, 8; \, P=0.03) \) and time of evaluation \( (F=7.54; \, df=4, \, 32; \, P<0.01) \) were significant for *Helicoverpa armigera* damage to fruiting forms (Figure 4.1). The interaction between cotton plant type and time of evaluation \( (F=3.22; \, df=4, \, 32; \, P=0.02) \) also was significant. Initial damage (3rd day) at the site of infestation was 18.4 (36.8%) and 12.6 (25.2%) fruiting forms per 50 plants on non-Bollgard and Bollgard® cotton, respectively. At 5th day on non-Bollgard cotton plant, *Helicoverpa armigera* damaged 30.9 fruiting forms per 50 plants consisting of 8.2 squares and 22.5 bolls. Cumulative damage increased to 40.3 fruiting forms per 50 plants for non-Bollgard cotton plant at 7th day (Figure 4.1).
Damaged fruiting forms consisted of 11.9 squares and 28.3 bolls. After 7th day, damage began to decrease on non-Bollgard® cotton plant because bollworms were beginning to complete larval development. At 9th day on non-Bollgard cotton plant, cumulative *Helicoverpa armigera* damage averaged 46.6 fruiting forms per 50 plants and consisted of 13.3 squares and 32.9 bolls (Figure 4). No additional damage was observed on 11th day. In this study Damage to white flowers was minimal. Additional damage after that at the site of infestation was observed on Bollgard® cotton plant; however, damage did not increase as rapidly as it did on non-Bollgard cotton plant. *Helicoverpa armigera* damaged a mean of 16.8 fruiting forms per 50 plants at 5th day (Figure 4.). Numbers of damaged squares and bolls ranged from 0.0 to 7.0 (µ=2.0) and 6.0 to 24.0 (µ=14.5), respectively. At 7th day, bollworms damaged a mean of 17.9 fruiting forms per 50 plants on Bollgard® cotton plant. Numbers of damaged squares and bolls per 50 plants ranged from 0.0 to 7.0 (µ=2.5) and 3.0 to 26.0 (µ=15.0), respectively. At 9th day, bollworm *Helicoverpa armigera* damaged a mean of 18.9 fruiting forms per 50 plants. Numbers of damaged squares and bolls ranged from 0.0 to 7.0 (µ=2.9) and 3.0 to 27.0 (µ=16.0), respectively.

At 5th day, a lower percentage of *Helicoverpa armigera* were recovered from Bollgard® (72.3%) cotton plant than non-Bollgard (97.5%) cotton plant plants (Figure 5.). After 5th day, the percentage of *Helicoverpa armigera* recovered from conventional cotton plant declined more rapidly than on Bollgard® cotton plant. Consequently, percentage recovery of larvae was similar between Bollgard® (59.1%) cotton plant and non-Bollgard® (56.7%) cotton plant at 7th day. At 9th day, a higher percentage of larvae recover from Bollgard® (31.6%) plants than on non-Bollgard (2.8%) plants. No larvae were recovered after 9th day on cotton plant variety. An individual bollworm damaged more squares ($F=45.18; df=1, 7; P<0.01$), bolls ($F=20.17; df=1, 7; P<0.01$), and total fruiting forms ($F=46.05; df=1, 7; P<0.01$) through all larval stadia on conventional cotton plant than on Bollgard® cotton plant (Figure 6.). On non-Bollgard cotton plant, 4.3 fruiting forms (2.9 bolls, 0.1 white flowers, and 1.3 squares) were damaged per larva.
On Bollgard® cotton plant, 2.7 fruiting forms (2.1 bolls, 0.1 white flowers, and 0.5 squares) were damaged per larva.

*Helicoverpa armigera* Damaged Fruiting Forms on Bollgard® and Bollgard® II Cotton plant.

Similar to the previous study, bollworm, *Helicoverpa armigera* damaged more fruiting forms on conventional cotton plant than on Bollgard® cotton plant. Also, bollworms damaged more fruiting forms on Bollgard® cotton plant than on Bollgard® II cotton plant. Effects for cotton type ($F=18.98; df=2, 15; P<0.01$) and time of evaluation ($F=26.14; df=4, 60; P<0.01$) for bollworm damage to fruiting forms were observed. A cotton plant type by time of evaluation ($F=9.89; df=8, 60; P<0.01$) interaction also was observed. At 3 d, bollworms, *Helicoverpa armigera* damaged 8.5 (85%), 6.3 (63%), and 5.7 (57%) bolls per 10 plants on non-Bollgard, Bollgard®, and Bollgard® II, respectively, at the site of infestation (Figure 7).

After the 3 day period, *Helicoverpa armigera* damaged fruiting forms increased rapidly on conventional cotton plant. At 5th day, *Helicoverpa armigera* damaged 18.2 fruiting forms per 10 plants on conventional cotton plant. Damaged fruiting forms included 5.8 squares and 12.0 bolls per 10 plants. At 7th day on conventional cotton plant, 23.6 fruiting forms (8.2 squares and 14.7 bolls) per 10 plants were damaged by *Helicoverpa armigera*. Bollworm, *Helicoverpa armigera* damage decreased after 7th day; however, 25 fruiting forms were damaged per 10 plants by *Helicoverpa armigera* at 9d on conventional cotton plant. Numbers of *Helicoverpa armigera* damaged squares and bolls averaged 9.0 and 15.3, respectively, on conventional cotton plant. No additional injury was observed after 9d on conventional cotton plant. Cumulative *Helicoverpa armigera* damage increased slightly after the initial 72hr period on Bollgard® cotton plant (Figure 7). At 5th day, *Helicoverpa armigera* larvae damaged nine fruiting forms per 10 plants consisting of 0.7 squares and 8.0 bolls. At 7d, bollworms damaged a mean of 1.0 square and 8.7 bolls for a total of 10.9 fruiting forms per 10 plants on Bollgard® cotton.
plant. At 9d, 11.5 fruiting forms per 10 cotton plants were damaged by *Helicoverpa armigera* on Bollgard® cotton plant. *Helicoverpa armigera* larvae damaged fruiting forms consisted of 1.3 squares and 9.0 bolls per 10 plants. No additional damage was observed after 9d on Bollgard® cotton plant. On Bollgard® II cotton plant, little damage was observed after the initial 72hr period (Figure 7).

At 5th day, cumulative *Helicoverpa armigera* larvae damage included 0.2 squares, 0.2 white flowers, and 6.0 bolls per 10 cotton plants. Only one larva of *Helicoverpa armigera* survived after 5d on Bollgard® II. The larva damaged one additional white flower and one additional boll, but did not complete development.

The percentage of *Helicoverpa armigera* larvae recovered from conventional and Bollgard® cotton plant followed a similar trend to that in the previous study (Figure 8). At 5th day, a lower percentage of *Helicoverpa armigera* larvae were recovered on Bollgard® (73.6%) cotton plant than on non-Bollgard (96.1%) cotton plant. At 7d, the percentages of *Helicoverpa armigera* larvae recovered were similar on Bollgard® (59.7%) cotton plant and conventional (67.8%) cotton plant. At 9d, a higher percentage of *Helicoverpa armigera* larvae remained on Bollgard® (40.3%) cotton plants compared to conventional (8.1%) cotton plants. No larvae were recovered after 9d on either variety. The percentage of *Helicoverpa armigera* larvae recovered on Bollgard® II declined very rapidly. At 5d, 25.0% of larvae were recovered. No bollworm larvae were recovered after 7d on Bollgard® II cotton plant.

An individual bollworm larva damaged more fruiting forms on conventional cotton plant (6.6) than on Bollgard® (3.5) or Bollgard® II (0.8) cotton plant during all larval stadia ($F=20.76; df=2, 12; P<0.01$) (Figure 9). Also, more fruiting forms were damaged by an individual *Helicoverpa armigera* larva on Bollgard® cotton plant than on Bollgard® II cotton plant. An individual *Helicoverpa armigera* damaged more squares on non-Bollgard cotton plant (2.6) than on Bollgard® (0.4) or Bollgard® II (<0.1) ($F=38.76; df=2, 12; P<0.01$). More white flowers were damaged by an individual *Helicoverpa armigera* larva on conventional cotton plant than on Bollgard® II cotton plant. An individual *Helicoverpa armigera* damaged more white flowers on non-Bollgard cotton plant (10.0) than on Bollgard® (2.6) or Bollgard® II (1.3) ($F=38.76; df=2, 12; P<0.01$). More white flowers were damaged by an individual *Helicoverpa armigera* larva on conventional cotton plant than on Bollgard® II cotton plant. An individual *Helicoverpa armigera* damaged more white flowers on non-Bollgard cotton plant (10.0) than on Bollgard® (2.6) or Bollgard® II (1.3) ($F=38.76; df=2, 12; P<0.01$).
armigera on Bollgard® cotton plant (0.5) than on non-Bollgard cotton plant (0.3) or Bollgard® II (0.1) cotton plant \((F=8.63; df=2, 12; P<0.01)\).

Also, more white flowers were damaged on conventional cotton plant than on Bollgard® II cotton plant. However, white flower damage was minimal during this study. An individual Helicoverpa armigera larva damaged fewer bolls on Bollgard® II (0.8) cotton plant than on conventional (3.4) or Bollgard® (2.7) cotton plant \((F=13.23; df=2, 12; P<0.01)\).

### Discussion

Bollworms, Helicoverpa armigera larvae damaged more fruiting forms on conventional cotton plant than on Bollgard® or Bollgard® II cotton plants. However, additional structures after those at the site of infestation were damaged \(P<0.01\) by Helicoverpa armigera larvae on Bollgard® cotton plants. Helicoverpa armigera larvae damage at 3 d on Rasi 2000 (non-Bollgard, 36.8%) and Rasi 134 (Bollgard, 25.2%) was lower than those observed by Gore et al. (2000a). Gore et al. (2000a) observed 69.7% and 48.5% boll abscission 72hr after infestation of Rasi 2000 and Rasi 134 white flowers, respectively. In contrast, initial damage on Rasi 2000 (non-Bollgard, 85.0%) and Rasi 134 (Bollgard, 63.0%) was higher than that observed by Gore et al. (2000a). Differences in damage between the two current studies may be attributed to differences in cotton plant variety. Also, environmental conditions may have been more conducive for Helicoverpa armigera feeding and survival during the Bollgard® II study because of the later planting date.

In general, Helicoverpa armigera damaged fruiting forms increased more rapidly on conventional cotton plants than on Bollgard® or Bollgard® II cotton plants. Helicoverpa armigera larvae damage on conventional cotton plant peaked at 7d. Although damage never reached the same levels on Bollgard® cotton as those observed on conventional cotton plant, some additional damage did occur. Therefore, insecticide applications may be required to control bollworms feeding in white flowers. To further support this,
Burd et al. (1999) found that yields of Bollgard® cotton plant were significantly improved following applications of a pyrethroid for the control of *Helicoverpa armigera* larvae. However, pyrethroids have a broad spectrum of activity and some of the yield increase may be attributed to control of other pests such as tarnished plant bugs, *Lygus lineolaris* (Palisot de Beauvois) or stink bugs (Hemiptera: Pentatomidae).

Gore et al. (2000b) determined that cotton plants compensate for relatively high levels of boll damage during the early flowering period and low to moderate levels of boll damage during later flowering periods. However, in that study, boll damage levels were applied at a specific point in time and did not consider additional damage over time or damage to other fruiting structures (squares and flowers). Therefore, based on the available data, insecticide applications may be warranted for control of *Helicoverpa armigera* in Bollgard® cotton plant, especially when other pests are present or when insect pressure persists over time. In contrast, little damage was observed on Bollgard® II cotton plant after the initial damage observed at the site of infestation. Therefore, insecticide applications targeting *Helicoverpa armigera* larvae may not always provide significant yield increases and thus may not be economical for Bollgard® II cotton plant. Numbers of damaged fruiting forms for non-Bollgard cotton plant were similar to those observed in previous studies (Adkisson et al. 1964, Anonymous 1967). Studies in Arkansas revealed that an individual *Helicoverpa armigera* larva damaged an average of 3.8 squares and 2.2 bolls (Anonymous 1967). *Helicoverpa armigera* damaged fewer fruiting forms on Bollgard® cotton plant compared to conventional cotton plants.

However, *Helicoverpa armigera* larvae on Bollgard® cotton plant fed for a longer period of time. Consequently, more *Helicoverpa armigera* larvae remained on Bollgard® cotton plants at 9 d, while on conventional cotton plant most of the *Helicoverpa armigera* larvae had left the plants to pupate. On Bollgard® II cotton plants, *Helicoverpa armigera* larvae damaged very few squares and white flowers. The majority of damage to the Bollgard® II cotton
plants line consisted of small bolls at the site of infestation.

In conclusion, these data support the application of insecticides to control *Helicoverpa armigera* larvae in Bollgard® cotton plant. Protein expression in Bollgard® plants varies both temporally and spatially and causes *Helicoverpa armigera* larvae injury to vary accordingly.