

Seasonal Pattern of Infestation by Spotted Bollworm, *Earias insulana* (Boisd.) and Pink Bollworm, *Pectinophora gossypiella* (Saund.) in Field Plots of Transgenic Bt and non-Bt Cottons

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Abstract.- A transgenic Bt cotton line IR-FH-901 carrying Cry1Ac toxic gene from *Bacillus thuringiensis* was evaluated for resistance against spotted bollworm *Earias insulana* (Boisd.) and pink bollworm *Pectinophora gossypiella* (Saund.) in Punjab province, Pakistan during 2007 and 2008. We found no significant differences in spotted bollworm egg density between Bt and conventional cotton but larval density was significantly lower in unsprayed Bt cotton than unsprayed non-Bt cotton plots and insecticides were applied in sprayed non-Bt plots to keep the pest population below the economic threshold level. The rosette flower density caused by pink bollworm infestation and number of pink bollworm larvae found in bolls of Bt cotton were significantly lower than non-Bt cotton plots. We conclude that transgenic Bt cotton is more effective in season-long control of spotted and pink bollworm.

Key words: Population dynamics, spotted bollworm, pink bollworm, Bt cotton, Pakistan

INTRODUCTION

Pakistan is the fourth-largest producer of cotton after China, the USA and India. Cotton is an important fiber and cash crop of Pakistan and known as “white gold”. The average yield of cotton is about 570.99 kg/ha, which is low as compared with other cotton growing area of the world (Bakhsh *et al.*, 2005). The low productivity of cotton is caused by many factors but the most serious one is the intensity of insect injury. Among the bollworm complex, the pink bollworm *Pectinophora gossypiella* (Saund.) and the spotted bollworm *Earias insulana* (Boisd.) are the most destructive in Pakistan and cause significant yield reduction (Abro *et al.*, 2004; Hamed and Nadeem, 2010), because of damage to flowers, squares and bolls (Gore *et al.*, 2000; Ashfaq *et al.*, 2006). It is estimated that in Pakistan, farmers spend US\$300 million on pesticides annually, of which more than 80% is used on cotton, especially for bollworms (Rao, 2007; Arshad and Suhail, 2011).

One option to reduce the insecticide use on cotton is the exploitation of transgenic Bt cotton as a

component of integrated pest management (Gore *et al.*, 2001). Bt cotton, the first transgenic non-food crop, has provided a specific, safe and effective tool for the control of lepidopteran pests (Shelton *et al.*, 2002; Mendelsohn *et al.*, 2003; Wu and Guo, 2005). Bt cotton expressing the Cry1Ac protein has been available commercially in the USA since 1996 and is also being grown in Mexico, Colombia, Australia, China, India, Argentina, and South Africa (James, 2006). The growing of transgenic cotton is however, a new technology in Pakistan agriculture and every new technology performs differently in different ecological systems. The present study was thus planned, to investigate the impact of transgenic Bt cotton on the seasonal infestation by pink and spotted bollworm under sprayed and non-sprayed conditions compared with conventional non-Bt cotton in field conditions.

MATERIALS AND METHODS

Study area and design

The field experiments were carried out for two cotton seasons, 2006 and 2007, at the Postgraduate Agriculture Research Station (PARS), Faisalabad, Punjab, Pakistan (31° 21.52 North and 72° 59.40 East), where wheat and cotton are commonly intercropped. The experimental fields

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were laid out in a randomized complete block design (RCBD) consisting of four treatments each with four replications. Each replicated plot was about 0.05 ha (hectare). A gap of 5-m was left between plots to avoid the influence of treatments on insect population in neighbouring plots (Men *et al.*, 2003). The transgenic Bt-cotton line, "IR-FH-901" (NIBGE, Faisalabad, Pakistan) producing the Cry1Ac insecticidal protein of *Bacillus thuringiensis* was compared with its parent non-transgenic, conventional cotton cultivar, "FH-901" (AARI, Faisalabad, Pakistan). The experimental area selected was isolated from other sprayed cotton to reduce the chances of insecticidal drift across the unsprayed area. Seeds were sown at a rate to produce an expected plant population of 60,000 per ha with row to row and plant to plant distance of 0.75 and 0.25 m respectively. The experimental fields were maintained according to the recommended agronomic practices. The insecticides used in sprayed plots were based on the economic thresholds level (ETL) for pink bollworm (before boll formation, 5% rosette flowers; at boll formation, 5% damage or 5 larvae per 100 bolls) and spotted bollworm (3 larvae/ 25 plants). Insecticides were sprayed with a knapsack sprayer and used hollow cone nozzles, held 0.3-0.5 m above the cotton plants (Wu *et al.*, 2002). The same insecticides were applied in both years, while no insecticides were applied in unsprayed plots (Table III). The experimental design and sampling procedures were similar in both years of field studies. The treatments included were; a) transgenic Bt-cotton without insecticide application (Bt cotton unsprayed); b) transgenic Bt-cotton with insecticide application (Bt cotton sprayed); c) non transgenic cotton without insecticide application (non-Bt cotton unsprayed); d) non transgenic cotton with insecticide application (non-Bt cotton sprayed).

Sampling

Plant inspection method was used and sampling was initiated at the seedling emergence and continued weekly until mid-October. This resulted in at least 18 sampling dates. Populations were recorded from 15 plants selected at random in each replicate plot. The sampling for spotted bollworm was done at eggs and larval stages,

because these stages are less mobile and more likely to be affected by insecticides or Bt toxic proteins (Head *et al.*, 2005). Pink bollworm larvae were estimated by counting all larvae inside 25 randomly collected green bolls (14-20 d old) per plot. Rosette flowers caused by the pink bollworm larvae were also counted on 15 plants selected randomly in each plot. A typical rosette flower contains pink bollworm larva. The mean eggs, larvae and rosette flowers incidence of target pests in Bt and non-Bt cotton were worked out on per 15 plants basis for each treatment.

Statistical analysis

All data on population dynamics in different treatments for both seasons were analyzed using analysis of variance (ANOVA) and means were separated by using Tukey's Honestly Significant Difference (HSD) test. All analyses were done using SPSS (SPSS Institute, Chocago, II., USA) and Statistica-6 software. The multifactor effects of year, variety and pesticide were analyzed using GLM procedure in Statistica-6 statistical software.

RESULTS

Seasonal abundance of spotted bollworm eggs and larvae

The results revealed that spotted bollworm eggs and larvae appeared in the field during the 2nd week of July through mid-October. The incidence of eggs varied during seasons and peaked on 13 August and 10 September in 2006 and 30 July and 17 September in 2007 (Fig. 1). The pooled data of all observations showed no significant ($P > 0.05$) differences in egg numbers between Bt and non-Bt cotton plots under sprayed and unsprayed conditions during the both seasons in 2006 ($F = 3.49$; $df = 3$; $P = 0.06$) and 2007 ($F = 2.70$; $df = 3$; $P = 0.09$) (Fig.2).

In 2006 larval populations peaked on 20 August and 17 September in unsprayed non-Bt cotton and were significantly ($P < 0.05$) higher than other treatments (Fig. 3A). In the second season (2007) larval populations were comparatively low and peak numbers were observed on 20 August, 10 and 24 September (Fig. 3B). The pooled data of all the observation revealed significant differences

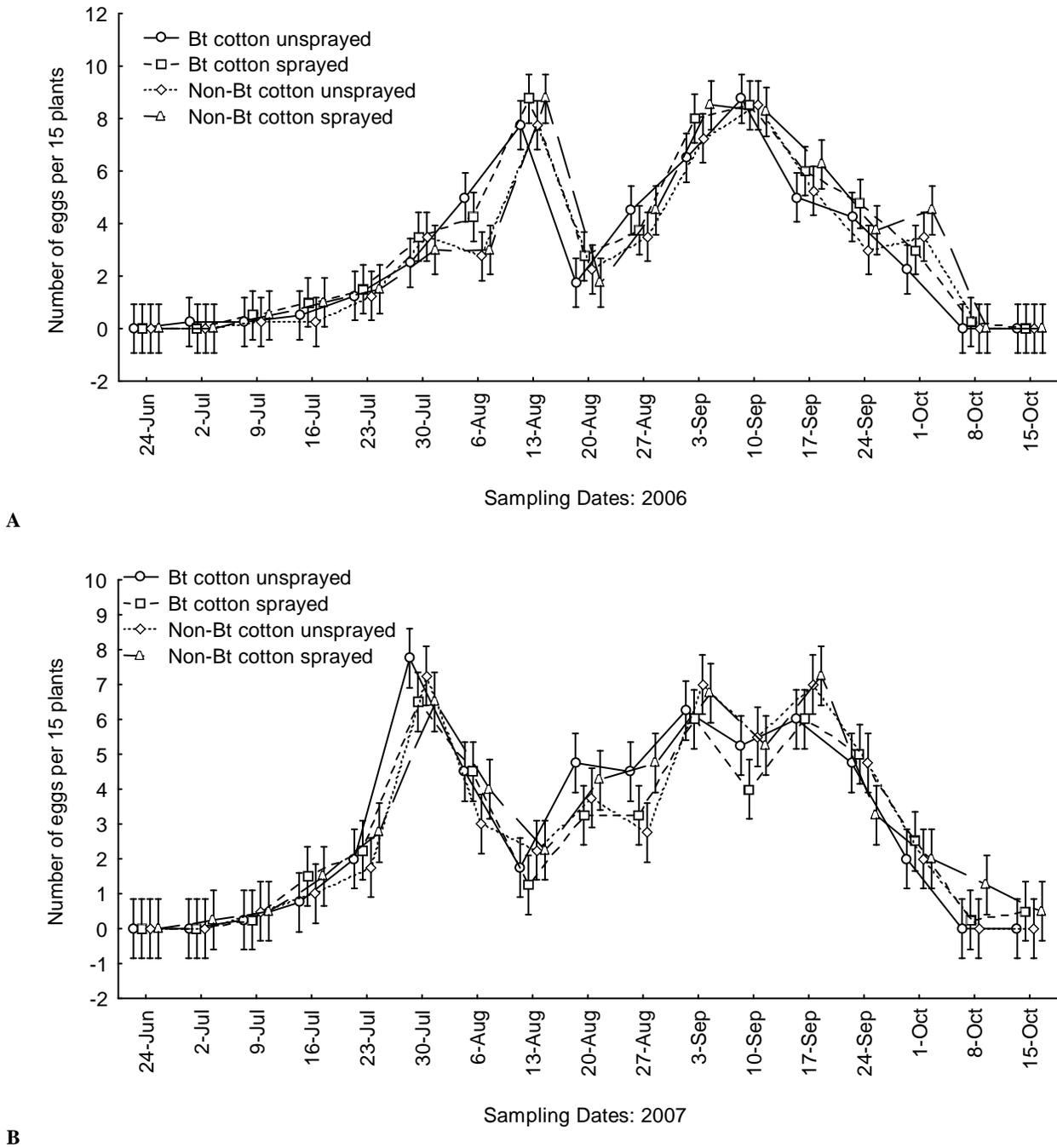


Fig. 1. Seasonal abundance (Mean \pm SE) of spotted bollworm eggs in Bt and non-Bt cotton, (a) 2006; (b) 2007.

($P < 0.05$) in larval populations among the treatments in 2006 ($F = 448.79$; $df = 3$; $P < 0.001$) and 2007 ($F = 686.23$; $df = 3$; $P < 0.001$). The larval population was lower in unsprayed Bt plots (1.17 and 1.00 larvae/15 plants in 2006 and 2007, respectively) and

were at par with sprayed Bt plots (1.22 and 0.94 larvae/15 plants), where no insecticide application was needed to control this pest. While, unsprayed non-Bt plots have significantly ($P < 0.05$) higher larval population (8.01 and 5.78 larvae/15 plants

respectively in 2006 and 2007) and insecticides were applied in sprayed non-Bt plots to keep the pest population below the economic threshold level (Fig. 4, Table I).

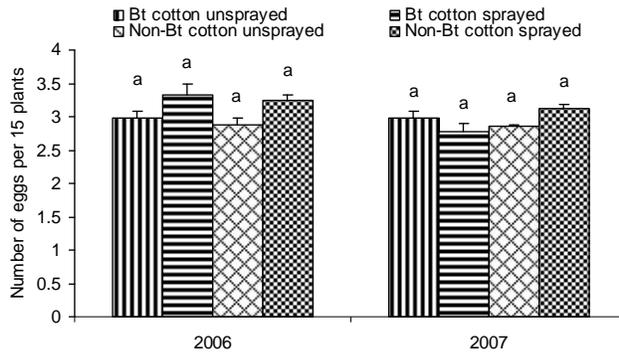


Fig. 2. Seasonal abundance (Overall mean \pm SE) of spotted bollworm eggs in 2006 and 2007, bars indicated by different letters are significantly different (Tukey's HSD, $P < 0.05$).

Table I.- Multi-factor effects of year, variety and pesticide on the mean seasonal abundance of spotted bollworm egg and larvae.

Factors	Egg F-values	Larvae F-values
Year	10.65*	98.35*
Variety	0.11	1541.56*
Pesticide	12.46*	626.85*
Year \times Variety	3.23	54.72*
Year \times Pesticide	8.98*	11.66*
Variety \times Pesticide	4.83*	622.79*
Year \times Variety \times Pesticide	4.26	15.87*

Significant difference indicated by * $P < 0.05$

A multi-factor (years, variety and pesticide) effects revealed that variety and interactions of years \times variety and all of three factors (year \times variety \times pesticide) had no significant ($P > 0.05$) differences in eggs number, while year, pesticide and their interactions were significant ($P < 0.05$). There were, however, the significant differences ($P < 0.05$) in larval numbers among the years, variety, pesticides and all their interactions (Table I).

Seasonal abundance of rosette flowers and pink bollworm larvae

The population density of pink bollworm was recorded by the number of rosette flowers and

number of larvae found in bolls. Rosette flowers density varied significantly in treatments throughout the season in 2006 and maximum numbers were observed on 30 July and 20 August in unsprayed non-Bt cotton. Curves of rosette flower dynamics in 2007 presented are shown in Fig. 5b. As in 2006, rosette flower numbers varied significantly during the season, which peaked in late August in unsprayed non-Bt cotton. A very few or no rosette flowers were observed in Bt cotton with and without spray (Fig. 5).

Larval densities peaked on 3 and 24 September in unsprayed non-Bt cotton in 2006 (Fig. 6A). Larval numbers also varied significantly among the treatments ($F = 420.95$; $df = 3$; $P < 0.001$) and were peaked on 27 August and 17 September in unsprayed non-Bt cotton (Fig. 6B). The overall data showed a significantly ($P < 0.05$) lower larval density (0.35 larvae/25 bolls) in unsprayed Bt cotton compared to unsprayed non-Bt cotton (2.45 larvae/25 bolls).

Table II.- Multi-factor effects of year, variety and pesticide on the mean seasonal abundance of pink bollworm.

Factors	Rosette flowers F-values	Larvae F-values
Year	18.31*	9.29*
Variety	548.46*	623.34*
Pesticide	64.22*	175.99*
Year \times Variety	11.19*	16.30*
Year \times Pesticide	6.59*	0.82
Variety \times Pesticide	37.78*	127.43*
Year \times Variety \times Pesticide	8.29*	1.53

Significant difference indicated by * $P < 0.05$

The pooled data of all the observations showed significant differences in rosette flowers ($F = 151.24$; $df = 3$; $P < 0.001$) and larval densities in bolls among the treatments ($F = 79.73$; $df = 3$; $P < 0.001$) (Fig. 7). Larval number increased in Bt cotton with and without spray in September and October, but remained below the economic threshold and needed no insecticide in sprayed plots of Bt cotton. The larval density was maximum in unsprayed non-Bt cotton (1.98/25 bolls) than unsprayed Bt cotton (0.46 larvae/ 25 boll). A summary of multi-factor analysis showed that all

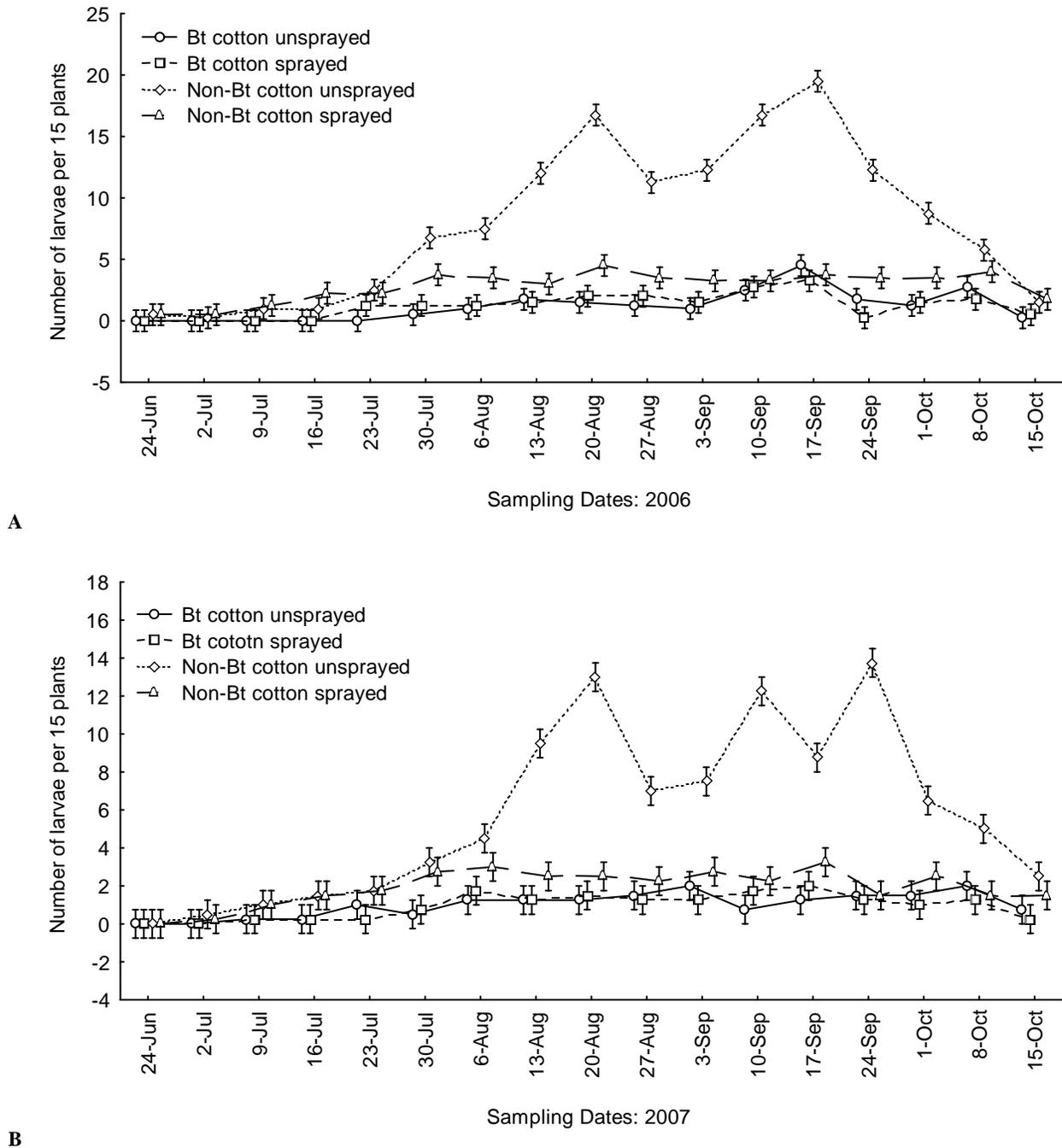


Fig. 3. Seasonal abundance (Mean \pm SE) of spotted bollworm larvae in Bt and non-Bt cotton, (a) 2006; (b) 2007.

factors (year, variety and pesticide) and their possible interactions were significant for rosette flowers and larval densities (Table II). No insecticide application was needed to control the

pink bollworm in sprayed Bt cotton, whereas insecticide applications were done in sprayed non-Bt cotton to keep the population below the economic threshold level (Table III).

Table III.- Insecticides application based on economic threshold level (ETL) in plots under sprayed conditions.

Insecticides	Target pest	Spraying				Dose rate /acre
		Non-Bt cotton sprayed		Bt cotton sprayed		
		2006	2007	2006	2007	
Bifenthrin 10 EC	Spotted bollworm	18 August	10 August	-	-	250 ml/acre
Thiodicarb 80 DF	Pink bollworm	28 August	21 August	-	-	480 gm/acre
Triazophos 40 EC	Spotted and pink bollworm	7 September	13 September	-	-	1000 ml/acre

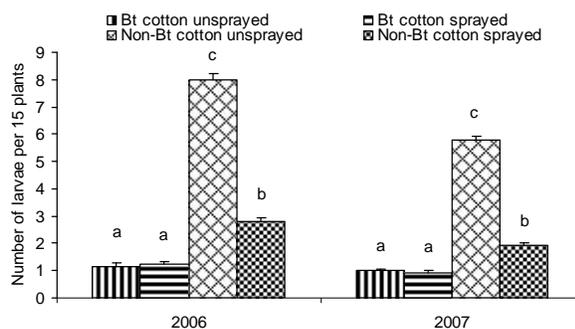


Fig. 4. Seasonal abundance (Overall mean \pm SE) of spotted bollworm larvae in 2006 and 2007, bars indicated by different letters are significantly different (Tukey's HSD, $P < 0.05$).

DISCUSSION

Seasonal abundance of spotted bollworm eggs and larvae

Spotted bollworm is an important pest of cotton causing damage to fruiting bodies and shedding of squares, flowers and bolls (Abro *et al.*, 2003; Ibargutxi *et al.*, 2006). Although, the primary aim of transgenic Bt cotton is to control the cotton bollworm, *H. armigera*, it also has a significant impact on other bollworm species, including *E. (delete arias) insulana* & *E. vitella* that are early to mid-season pests in cotton and hence transgenic Bt cotton can effectively control this pest during early-mid phase of the crop, when toxin expression is high (Kranthi *et al.*, 2004). Baseline susceptibility data has shown that Cry1Ac was highly toxic to spotted bollworm with an LC_{50} that ranged from 0.006 to 0.105 $\mu\text{g/ml}$ of diet and 0.88 ng/cm^2 for leaf-dip bioassays (Kranthi *et al.*, 2004). The results of the present study showed that Bt cotton significantly suppressed the larval population in sprayed and unsprayed Bt plots and hence no insecticide

application was needed against this pest in Bt cotton. These results agree with Kumar and Stanley (2006). An overall pooled data of all observations also revealed significantly lower larval populations in Bt cotton compared to non-Bt cotton. Similar results have been found by (Abro *et al.*, 2004; Morse *et al.*, 2005; Ashfaq *et al.*, 2006; Bal and Dhawan, 2008). Egg numbers were, however, not significant between treatments, which is in agreement with Kengegowda *et al.* (2005).

Seasonal abundance of rosette flowers and pink bollworm larvae

An overall pest population density of pink bollworm was low as compared with other target pests throughout both seasons. Results indicated, however, a lower density of rosette flowers and larvae in Bt cotton with and without spray than in non-Bt conventional cotton with and without spray. Similar results were obtained by Manjunatha *et al.* (2009) who reported significantly lower percent of rosette flowers (0.01-1.57%) in Bt cotton as compared to non-Bt cotton hybrids (8.72-11.57%) throughout the season. The present findings are also in corroboration with the results of Nadaf and Gould (2007) who reported significantly lower per cent of rosette flowers in Bt cotton (3.01 %) compared to non-Bt cotton (7.21 %). Further Douglas *et al.* (1992) reported that early in the season before bolls were available, the number of rosette flowers were 95 per cent lower in Bt cotton than non-Bt cotton. Larval populations were significantly higher in unsprayed non-Bt cotton in both seasons and an average 1-2 insecticide applications were used to control this pest. In general, larval density was higher in non-Bt cotton than those of Bt cotton. These results corroborate previous studies on pink bollworm infestation in Bt cotton (Arif *et al.*, 2006).

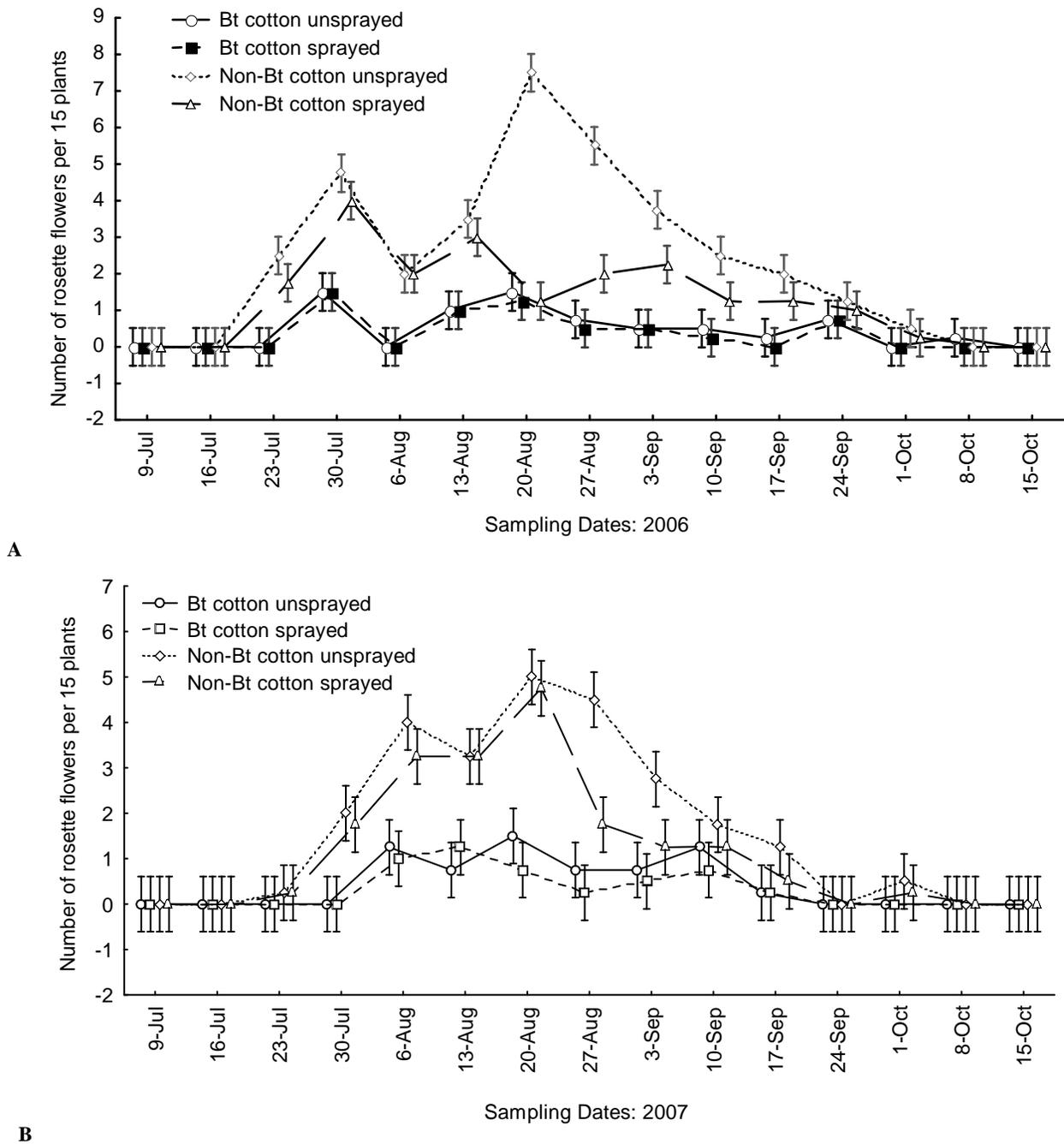


Fig. 5. Seasonal abundance (Mean \pm SE) of rosette flowers in Bt and non-Bt cotton, (a) 2006; (b) 2007.

The study indicated that some larvae survived in Bt cotton, late in the season (end of September and October). These results are in agreement with Wan *et al.* (2004). It may be due to the decreased Bt toxin expression at lateral stage of plant (Zhang *et al.*,

2001). It is evident, however, that Bt cotton effectively suppressed the larval density in early season to an extent that the pest could not cause economic damage in the late season. These late season larvae were below the economic threshold

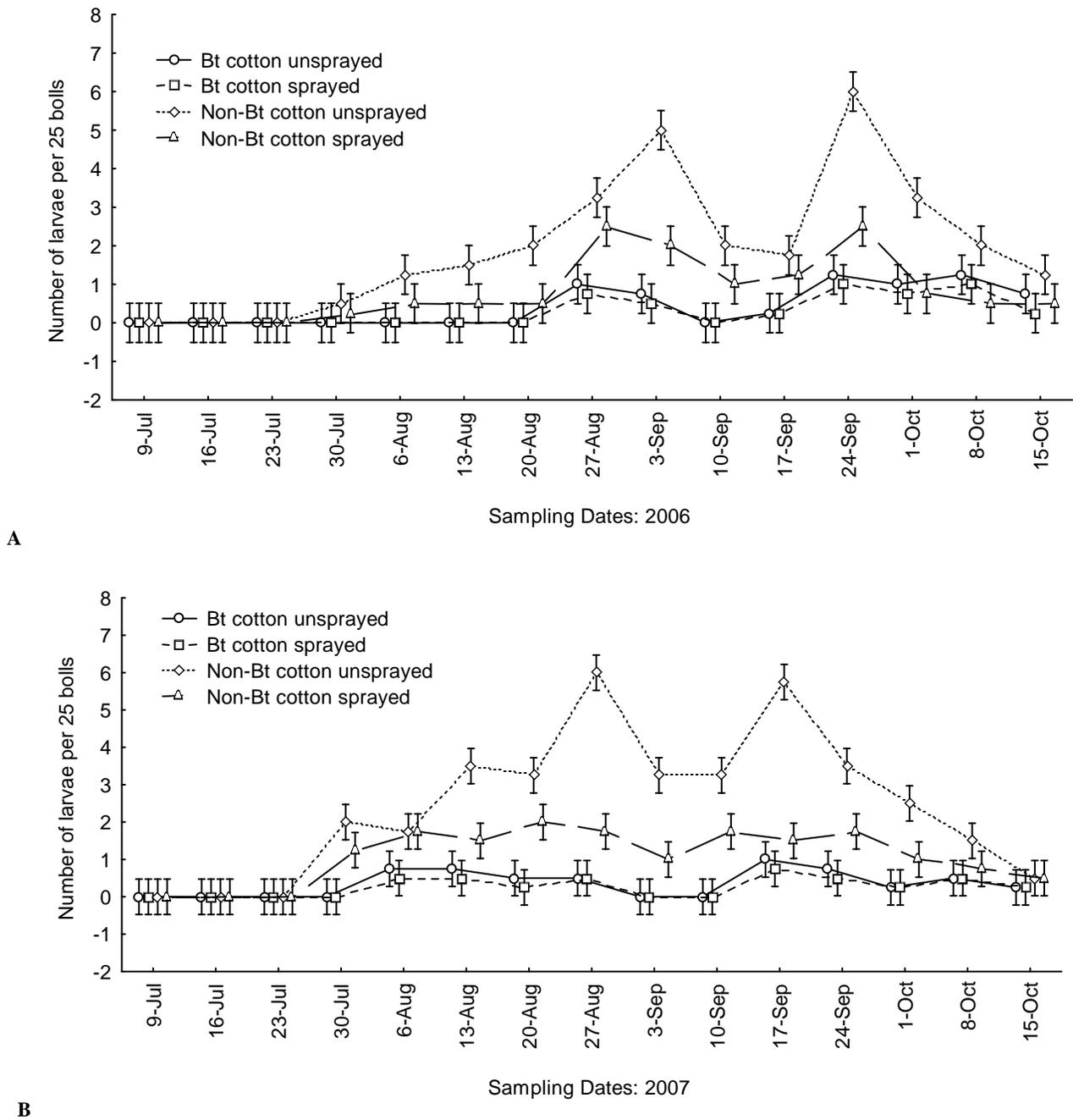


Fig. 6. Seasonal abundance (Mean \pm SE) of pink bollworm larvae in Bt and non-Bt cotton, (a) 2006; (b) 2007.

level and did not cause the economic damage. The results of the present study and those of other investigators (Liu *et al.*, 2001; Lavekar *et al.*, 2004; Nadaf and Gould, 2007) support the efficacy of Bt cotton for pink bollworm control.

In conclusion, the results of the present

studies show that transgenic Bt cotton line IR-FH-901 provide the protection against the infestation of spotted and pink bollworms. Our results provide the baseline information on the efficacy of transgenic Bt cotton lines for commercial planting in the cotton growing areas of Pakistan.

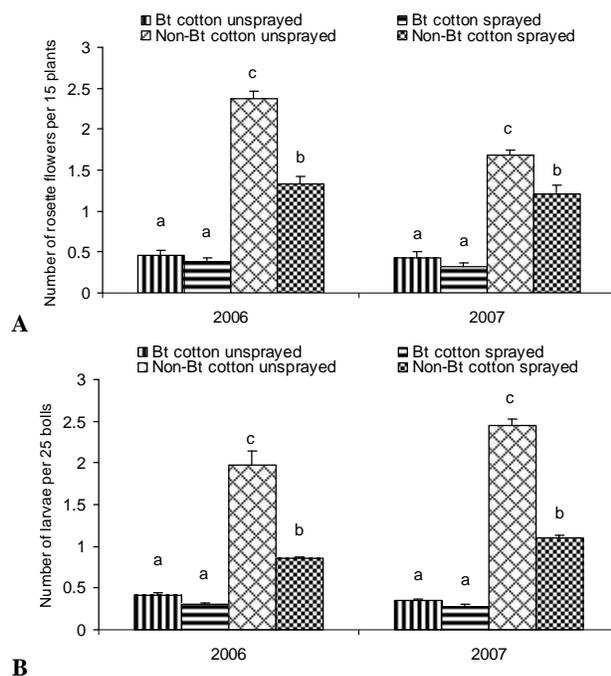


Fig. 7. Seasonal abundance (Overall mean \pm SE) of pink bollworm (a) rosette flowers (b) larvae in 2006 and 2007, bars indicated by different letters are significantly different (Tukey's HSD, $P < 0.05$).

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