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**Impact of *Bt* transgenic cottons and insecticides on target and nontarget insect pests, natural enemies and seedcotton yield in India**

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**ABSTRACT**

Genetically engineered cottons expressing  $\delta$ -endotoxins from *Bacillus thuringiensis* have been adopted on a large-scale worldwide. Therefore, we studied the efficacy of *Bt* cottons for the management of bollworms, their effects on nontarget insects, and seedcotton yield under

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insecticide protected and unprotected conditions. *Helicoverpa armigera* and *Earias vittella* damage was significantly lower in *Bt* than in non-*Bt* cottons, while no significant differences were observed in egg-laying by *H. armigera*. The populations of major nontarget sucking insect pests such as *Amrasca biguttula biguttula*, *Bemisia tabaci*, *Aphis gossypii*, *Oxycarenus laetus*, *Dysdercus koenigii*, and *Nezara viridula*; and the generalist predators viz., *Cheilomenes sexmaculatus*, *Chrysopa* spp., and spiders did not differ significantly between *Bt* and non-*Bt* cottons. Insecticide application resulted in resurgence of cotton aphid and whitefly, possibly because of elimination of natural enemies or better growth of plants under protected conditions. Abundance of bollworms, nontarget pests, and generalist predators was significantly greater before insecticide sprays than after insecticide application, except in a few cases. Bollworm damage was lower and seedcotton yields higher in *Bt* than in non-*Bt* cottons. The present studies indicated that *Bt* cotton hybrids are effective for the management of bollworms and yield more, and do not have any adverse effects on the abundance of generalist predators.

**Key words:** *Bacillus thuringiensis*, transgenic, cotton, bollworms, sucking insects, predators, nontarget effects, insecticides

**Short title for folio heading:** Nontarget effects of *Bt*-transgenic cotton

## INTRODUCTION

Genetically modified plants expressing *Bacillus thuringiensis* (*Bt*)  $\delta$ -endotoxin genes have been developed for resistance to insect pests, and some of them have been deployed successfully on a commercial scale for pest management (Sharma *et al.* 2004). Transgenic cotton and maize with resistance to lepidopteran insects have been released for cultivation in several countries, and were grown on more than 48 million ha worldwide in 2010. India ranks first in the world having 11.1 m ha area under *Bt*-cotton in 2011 (>90% of total cotton area in India), followed by China and USA (James 2011). Although, apparent benefits of cultivation of *Bt*-transgenic cotton have been observed in terms of significant reduction in insecticide usage, particularly against bollworms, increased yields, and reduced production costs and environmental contamination (Edge *et al.* 2001, Shelton *et al.* 2002, Sharma and Pampapathy 2006). However, due to large-scale adoption of *Bt* cottons, there might be putative risks such as loss of susceptibility to *Bt* toxins in the target pests, effects on nontarget organisms, altered biodiversity, and disruption of ecosystem processes (Wolfenbarger and Phifer 2000, Kranthi and Kranthi 2004, Sisterson *et al.* 2004, Sharma *et al.* 2007, Dhillon and Sharma 2010), which are equally important and need greater attention and continued monitoring of such effects, if any.

Considerable information has been generated on the relative efficacy of transgenic cottons against the target and nontarget insects on a long-term basis in USA, Australia, and China (Naranjo 2009), but there is little information on such effects of *Bt* cotton on nontarget insect pests and natural enemies in the tropics (Qaim and Zilberman 2003). Moreover, the information on comparative biosafety of insecticides and *Bt*-transgenic crops to non-target arthropods is very limited under Indian conditions. The cropping systems in tropics are quite diverse, and consist of several crops that serve as alternate and collateral hosts of the major pest, *Helicoverpa armigera* (Hubner), and other nontarget insect pests. Because of the multiplicity of crops and cropping systems, the performance and interactions of transgenic crops in different agro-ecosystems are likely to be quite complex. Also the issue of insecticide abuse and their adverse effects on insect diversity, pest resurgence, and natural

enemies is a major concern. Therefore, it is important to generate such information to take informed decisions about the impact of insecticide applications, *Bt*-transgenic crops, and the crop genotypes on the relative abundance of target and nontarget insect pests and their natural enemies. Therefore, the present studies were undertaken to compare the abundance of target and nontarget insect pests, generalist predators, bollworm damage, and seedcotton yield in *Bt*-transgenic and non-transgenic cottons under insecticide protected and unprotected conditions under field conditions. Such an information will be useful to compare relative adverse effects of deployment of transgenic crops vis-a-vis insecticide use in the ecosystem for sustainable crop production.

## **MATERIALS AND METHODS**

### ***Plant material***

Four *Bt*-transgenic cotton hybrids viz., MECH 12, MECH 162, MECH 184 (Mahyco Seeds Ltd., India), and RCH 2 (Rasi Seeds Ltd., India), and their non-transgenic counterparts were grown under field conditions on deep black soils (Vertisols) during the 2005-06, 2006-07, and 2007-08 cropping seasons at the International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India. Although, the bollgard II (BG II) was introduced in 2006, the studies were continued with the same set of *Bt*-hybrids to generate long-term information and gain a better understanding of the interactions involved. The seeds of each genotype were sown in four row plots of 4 m length on ridges at 75 cm apart with plant-plant spacing of 50 cm. There were three replications in split-plot design. The crop was raised under rain-fed conditions. Normal agronomic practices were followed for raising the crop (basal fertilizer N: P: K:: 100: 40: 60 kg ha<sup>-1</sup>). One set of the *Bt* and non-*Bt* cotton hybrids was fully protected (seed treatment + need based insecticide application), while another set was kept as an untreated control. The seeds of the cotton hybrids in protected plots were treated with imidacloprid 70WS @ 2 g per kg of seed. Six insecticide sprays were applied during the 2005-06 (methomyl 25SP, monocrotophos 36SL, methomyl 25SP, cypermethrin 25EC, monocrotophos 36SL, and methomyl 25SP), and five sprays each during the 2006-07 (monocrotophos 36SL, methomyl 25SP, endosulfan 35EC, cypermethrin 25EC, and methomyl 25SP) and 2007-08 (methomyl 25SP, methomyl 25SP, monocrotophos 36SL, methomyl 25SP, and cypermethrin 25EC) cropping seasons at fortnightly intervals starting from 75 days after seedling emergence (DAE) to 135 DAE. The insecticides were selected based on the severity of insect pests in the counterpart non-*Bt* cotton hybrids in the experimental plots and to some extent mimic the conditions of insecticide use prior to release of *Bt*-cotton, to gain a better understanding of the implications of insecticide use on natural enemy fauna in the ecosystem. Methomyl, monocrotophos, cypermethrin, and endosulfan were sprayed @ 500, 1000, 40, and 700 g a.i. h<sup>-1</sup>, respectively.

### ***Influence of Bt-transgenic cotton on abundance of target and nontarget insect pests and the generalist predators***

The abundance of target insect pests [cotton bollworm, *H. armigera*; and spotted bollworm, *Earias vittella* (Fab.)], nontarget insect pests [cotton leafhopper, *Amrasca biguttula biguttula* (Ishida); white fly, *Bemisia tabaci* (Gennadius); ash weevils, *Mylloceris* spp.; cotton aphid, *Aphis gossypii* Glover; dusky cotton bug, *Oxycarenus laetus* Kirby; red cotton bug, *Dysdercus koenigii* (Fab.); and green bug, *Nezara viridula* Linn.], and the generalist predators [coccinellid, *Cheilomenes sexmaculatus* Fab.; chrysopids, *Chrysopa* spp.; and spiders] was recorded from *Bt*-transgenic and non-transgenic cottons on five randomly tagged plants in the middle two rows of each plot at fortnightly intervals between 30 to 135 DAE. Observations

were also recorded on the target and nontarget insects, and the generalist predators before (24 h before spray) and after (48 h after spray) insecticide sprays.

The numbers of *H. armigera* eggs and larvae were expressed as eggs or larvae plants<sup>-10</sup>, while shoot damage by the spotted bollworm was recorded as percentage of plants with shoot damage. The cotton leafhopper and white fly adults and nymphs were recorded on the undersurface of the top five fully expanded leaves, and the data were expressed as numbers of leafhoppers or whiteflies plants<sup>-10</sup>. The cotton aphid infestation was expressed as percent aphid infested plants. Since the populations of dusky cotton bugs, red cotton bugs, green bugs, and ash weevils were low, the data were expressed as numbers of insects plants<sup>-100</sup>. The effect of *Bt*-transgenic plants on the activity and abundance of generalist predators was assessed by counting the numbers of coccinellid eggs, grubs and adults; chrysopid eggs and grubs; and the spiders on tagged plants as mentioned above. The data on coccinellids and chrysopids were expressed as eggs, grubs, or adults plants<sup>-100</sup>, while the spiders were expressed as numbers plants<sup>-10</sup>.

### ***Effects of Bt-transgenic cottons on bollworm damage and seedcotton yield***

The data were recorded on total numbers of green and mature bolls, and those damaged by bollworms [*H. armigera*, *E. vittella*, and *Pectinophora gossypiella* (Saunders)] on the five plants tagged at random. There was no infestation of pink bollworm, *P. gossypiella* on *Bt* and non-*Bt* cotton hybrids during the study period, and hence data on pink bollworm has been excluded from the analysis. Seedcotton was picked-up manually twice from each plot, dried in the sun and weighed, and expressed as kg ha<sup>-1</sup>.

### ***Statistical analysis***

The data were subjected to analysis of variance (ANOVA) using a factorial analysis, considering *Bt* versus non-*Bt*, and protected versus unprotected as the main and sub-treatments, using GenStat® 10<sup>th</sup> version statistical analysis program (Genstat 2008). The significance of differences between the treatments and their interactions were judged by F-test at  $P \leq 0.05$ , and the treatment means were compared by least significant difference (LSD) at  $P \leq 0.05$ .

## **RESULTS AND DISCUSSION**

### ***Influence of Bt-transgenic cottons on abundance of target insect pests***

There were no significant differences in *H. armigera* egg laying between *Bt*-transgenic and non-transgenic cottons ( $F_{1, 636} = 1.43$ ,  $P = 0.231$ ) (Figure 1a), indicating that the *H. armigera* adults do not discriminate between *Bt*-transgenic and non-transgenic cotton. The numbers of eggs laid by *H. armigera* were significantly more on insecticide protected plants as compared to that on the unprotected plants ( $F_{1, 636} = 29.72$ ,  $P < 0.001$ ) (Figure 1b), and their density was greater before insecticide application than after the insecticide sprays ( $F_{1, 636} = 31.51$ ,  $P < 0.001$ ) (Figure 1c). The numbers of *H. armigera* larvae were significantly more in non-*Bt* than in *Bt* cottons ( $F_{1, 636} = 108.97$ ,  $P < 0.001$ ) (Figure 1a). The insecticide protected plants had lower numbers of *H. armigera* larvae as compared to unprotected plants ( $F_{1, 636} = 8.11$ ,  $P = 0.004$ ) (Figure 1b). The numbers of *H. armigera* larvae before insecticide application were significantly more than after the insecticide sprays ( $F_{1, 636} = 13.96$ ,  $P < 0.001$ ) (Figure 1c). The spotted bollworm damage was significantly lower in *Bt*-transgenic than in the non-transgenic cottons ( $F_{1, 636} = 28.97$ ,  $P < 0.001$ ) (Figure 1a), but there was no influence of insecticide protection on spotted bollworm damage could be observed as no insecticide sprays were applied during the vegetative phase (Figure 1b, 1c). Interaction effects of *Bt*-transgenic cottons  $\times$  protection regimes  $\times$  insecticide sprays for numbers of *H. armigera* eggs and larvae, and the spotted bollworm damage were nonsignificant (Table 1). A significant

reduction in bollworm damage in *Bt*-transgenic cotton in combination with insecticide application has earlier been reported by Sharma and Pampapathy (2006).

### ***Influence of Bt-transgenic cottons on abundance of non-target insect pests***

There were no significant differences in numbers of cotton leafhoppers, *A. biguttula biguttula* ( $F_{1, 636} = 0.00$ ,  $P = 0.981$ ) and whiteflies, *B. tabaci* ( $F_{1, 636} = 0.00$ ,  $P = 0.955$ ) between *Bt*-transgenic and non-transgenic cottons (Figure 2a). However, insecticide protection had a significant influence on the population of *A. biguttula biguttula* and *B. tabaci* (Table 1). The numbers of *A. biguttula biguttula* were significantly greater on unprotected than on the protected plants, while reverse was true in case of *B. tabaci* (Figure 2b). Insecticide application reduced the numbers of cotton leafhoppers and the whiteflies significantly (Figure 2c). The percentage plants infested with *A. gossypii* were statistically similar in *Bt* and non-*Bt* cottons ( $F_{1, 636} = 0.09$ ,  $P = 0.767$ ) (Figure 2a), and *A. gossypii* infestation was significantly reduced after insecticide application ( $F_{1, 636} = 182.85$ ,  $P < 0.001$ ) (Figure 2c). Aphid infestation was greater in insecticide protected than the unprotected plants ( $F_{1, 636} = 21.98$ ,  $P < 0.001$ ) (Figure 2b), indicating resurgence of cotton aphid due to insecticide application. There were no significant differences in numbers of ash weevils ( $F_{1, 636} = 0.84$ ,  $P = 0.360$ ) and red cotton bugs ( $F_{1, 552} = 1.74$ ,  $P = 0.187$ ) between *Bt* and non-*Bt* cottons (Figure 2a). However, protection regimes showed a significant influence on the abundance of red cotton bugs and ash weevils (Table 1). Numbers of ash weevils and the red cotton bugs were significantly more on unprotected plants and before insecticide sprays than in plots protected with insecticide sprays (Figure 2b, 2c). There were no significant differences in numbers of dusky cotton bugs and green bugs on *Bt* and non-*Bt* cottons (Figure 2a), both before and after insecticide sprays (Figure 2c). However, protection regimes showed a significant influence on numbers of dusky cotton bugs ( $F_{1, 552} = 23.92$ ,  $P < 0.001$ ) and green bugs ( $F_{1, 636} = 129.91$ ,  $P < 0.001$ ). The numbers of dusky cotton bugs and green bugs were significantly greater in unprotected than in insecticide protected cottons (Figure 2b). The *Bt* × protection × spray interaction effects on the abundance of cotton leafhoppers, whiteflies, aphid infestation, ash weevils, red cotton bugs, dusky cotton bugs, and green bugs were nonsignificant (Table 1). Similar abundance of leafhoppers, whiteflies, aphids, ash weevils, red cotton bugs, dusky cotton bugs, and green bugs on *Bt* and non-*Bt* cottons could be due to their insensitivity to Cry1Ac toxin expressed in the *Bt*-cotton hybrids. Increased abundance of mirids, whiteflies, and leafhoppers; and a decrease in aphid infestation in *Bt* cotton have also been reported earlier (Wu *et al.* 2002, Wu and Guo 2003). Resurgence of some insect species in insecticide protected plots may be due to reduced numbers of predators, as no apparent effects of *Bt* have been observed on development and survival of *A. gossypii* when reared on *Bt*-cotton (Liu *et al.* 2005). Earlier studies have also reported negative effects of insecticides on insect communities in both *Bt*-transgenic and non-transgenic crops (Whitehouse *et al.* 2005, Head *et al.* 2005, Cattaneo *et al.* 2006, Naranjo 2009).

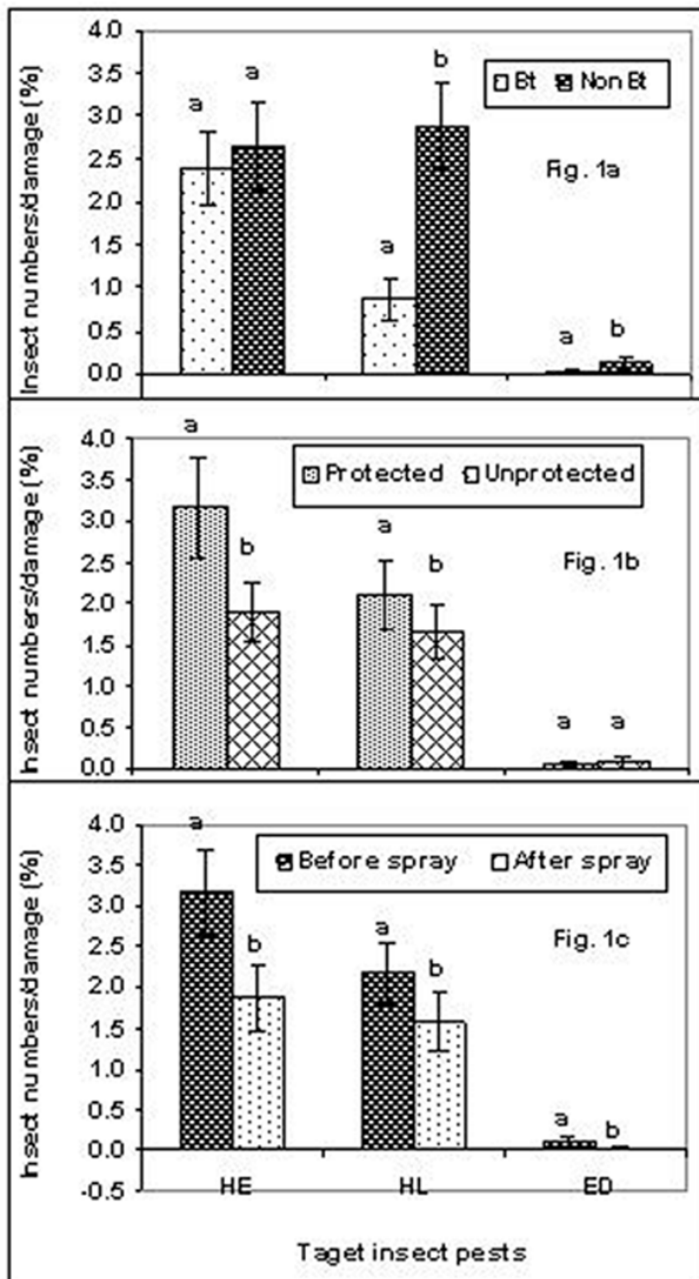


Figure 1. Mean numbers/damage by target insect pests ( $\pm$  SE) in *Bt*-transgenic and non-transgenic cottons (1a), under protected and unprotected (1b), and before and after insecticide sprays (1c). HE = *Helicoverpa armigera* eggs plants<sup>-10</sup>. HL = *Helicoverpa armigera* larvae plants<sup>-10</sup>. ED = *Earias vittella* damage (%). The paired bars following same letter are nonsignificant at  $P \leq 0.05$ .

Table 1. Abundance of target and nontarget insect pests, and natural enemies in *Bt*-transgenic and non-transgenic cottons under protected and unprotected conditions

Target/nontarget insects/ natural enemies	Before spray				After spray				LSD ( $P = 0.05$ ) for comparing			
	<i>Bt</i>		Non- <i>Bt</i>		<i>Bt</i>		Non- <i>Bt</i>		<i>Bt</i>	Protect	Spray	$Bt \times$ $P \times S$
	P	UP	P	UP	P	UP	P	UP				
<b>Target insect pests</b>												
Bollworm eggs plants <sup>-10</sup>	3.9 ± 0.6	2.2 ± 0.4	4.0 ± 0.7	2.6 ± 0.4	2.2 ± 0.5	1.3 ± 0.2	2.5 ± 0.6	1.5 ± 0.4	NS	0.45**	0.45**	NS
Bollworm larvae plants <sup>-10</sup>	1.1 ± 0.3	0.8 ± 0.2	4.4 ± 0.6	2.4 ± 0.4	0.6 ± 0.2	1.0 ± 0.3	2.3 ± 0.6	2.4 ± 0.4	0.36**	0.36**	0.36**	NS
Spotted bollworm damage (%)	0.01 ± 0.01	0.03 ± 0.01	0.2 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.01 ± 0.1	0.03 ± 0.02	0.04 ± 0.02	0.04**	NS	0.04**	NS
<b>Non-target insect pests</b>												
Cotton leafhoppers plants <sup>-10</sup>	36.5 ± 3.1	46.3 ± 3.6	36.5 ± 3.0	48.4 ± 3.7	13.4 ± 1.8	58.2 ± 5.1	13.1 ± 1.9	59.2 ± 4.9	NS	2.84**	2.84**	NS
Whiteflies plants <sup>-10</sup>	10.2 ± 1.5	4.6 ± 0.6	9.8 ± 1.5	4.8 ± 0.6	6.0 ± 1.0	4.8 ± 0.8	6.2 ± 1.0	5.1 ± 0.7	NS	0.98**	0.98**	NS
Aphids infested plants (%)	20.9 ± 3.5	14.7 ± 3.2	21.7 ± 3.6	15.8 ± 3.2	6.4 ± 2.8	0.8 ± 0.3	6.3 ± 2.7	0.5 ± 0.3	NS	2.35**	2.35**	NS
Ash weevils plants <sup>-100</sup>	16.8 ± 2.1	41.8 ± 5.0	14.9 ± 2.2	37.2 ± 5.0	0.7 ± 0.3	31.0 ± 4.9	0.2 ± 0.1	31.8 ± 5.0	NS	3.50**	3.50**	NS
Green bug plants <sup>-100</sup>	1.0 ± 0.3	6.7 ± 1.5	0.8 ± 0.3	7.9 ± 2.0	0.0 ± 0.0	11.0 ± 2.6	0.0 ± 0.0	8.2 ± 2.0	NS	1.30**	1.30**	NS
Red cotton bug plants <sup>-100</sup>	0.1 ± 0.1	13.9 ± 0.8	0.2 ± 0.1	22.4 ± 12.4	0.0 ± 0.0	9.5 ± 4.6	0.0 ± 0.0	10.4 ± 5.1	NS	4.80**	4.80**	NS
Dusky cotton bug plants <sup>-100</sup>	0.1 ± 0.1	0.5 ± 0.4	0.2 ± 0.2	1.4 ± 0.8	0.0 ± 0.0	25.4 ± 10.1	0.0 ± 0.0	33.8 ± 15.7	NS	6.80**	6.80**	NS
<b>Natural enemies</b>												
Chrysopid eggs plants <sup>-100</sup>	19.2 ± 4.0	9.0 ± 1.8	15.5 ± 3.1	10.2 ± 2.0	4.1 ± 1.3	8.4 ± 3.7	7.3 ± 3.0	10.9 ± 3.7	NS	NS	3.00**	NS
Chrysopid grubs plants <sup>-100</sup>	0.9 ± 0.3	0.2 ± 0.2	0.6 ± 0.2	0.3 ± 0.1	0.2 ± 0.2	2.9 ± 1.4	0.1 ± 0.1	1.3 ± 0.7	NS	0.40**	0.40*	NS
Coccinellid adults plants <sup>-100</sup>	13.6 ± 2.2	18.9 ± 2.3	11.0 ± 1.8	18.2 ± 2.1	0.9 ± 0.4	14.4 ± 2.8	1.1 ± 0.5	11.8 ± 2.3	NS	2.00**	2.00**	NS
Coccinellid eggs plants <sup>-100</sup>	0.5 ± 0.2	0.4 ± 0.2	0.8 ± 0.3	0.9 ± 0.4	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	NS	NS	0.30**	NS
Coccinellid grubs plants <sup>-100</sup>	3.4 ± 1.0	5.1 ± 1.7	2.9 ± 0.9	4.2 ± 1.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	NS	1.00**	1.00**	NS
Spiders plants <sup>-10</sup>	5.9 ± 0.7	14.0 ± 0.9	6.0 ± 0.7	14.7 ± 0.9	1.2 ± 0.3	17.4 ± 1.1	1.2 ± 0.3	17.8 ± 1.0	NS	0.68**	0.68*	NS

\*, \*\* = Significant at  $P \leq 0.05$ , and 0.01, respectively. NS = Nonsignificant at  $P \leq 0.05$ . P = Protected. UP = Unprotected. *Bt* = *Bt*-transgenic. Non-*Bt* = Non-transgenic. Spray = Comparison between before and after insecticide sprays.

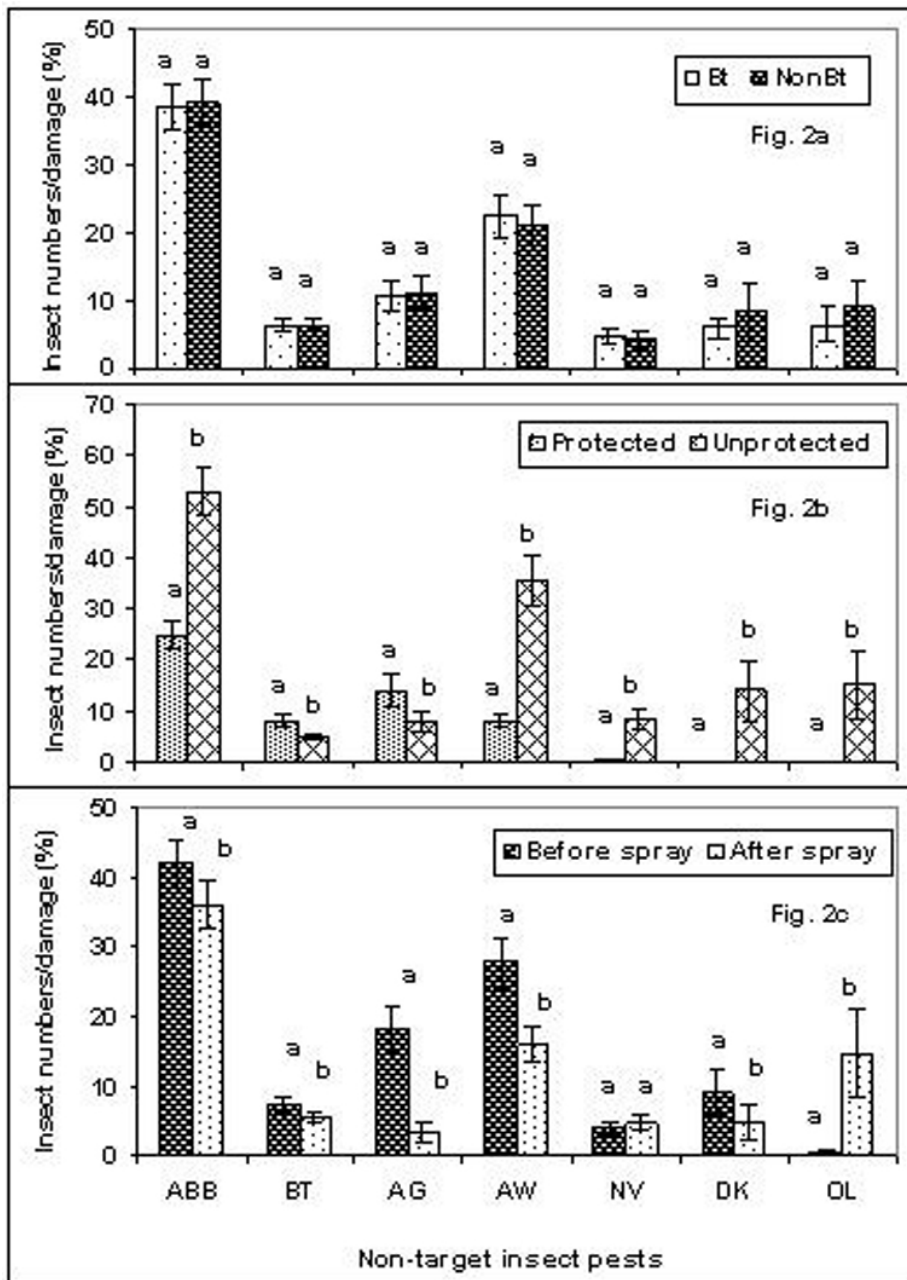


Figure 2. Mean numbers of nontarget insect pests ( $\pm$  SE) in *Bt*-transgenic and non-transgenic cottons (2a), under protected and unprotected (2b), and before and after insecticide sprays (2c). ABB = Cotton leafhoppers plants<sup>-10</sup>. BT = Whiteflies plants<sup>-10</sup>. AG = Plants with aphid infestation (%). AW = Ash weevils plants<sup>-100</sup>. NV = Green bugs plants<sup>-100</sup>. DK = Red cotton bugs plants<sup>-100</sup>. OL = Dusky cotton bugs plants<sup>-100</sup>. The paired bars following same letter are nonsignificant at  $P \leq 0.05$ .



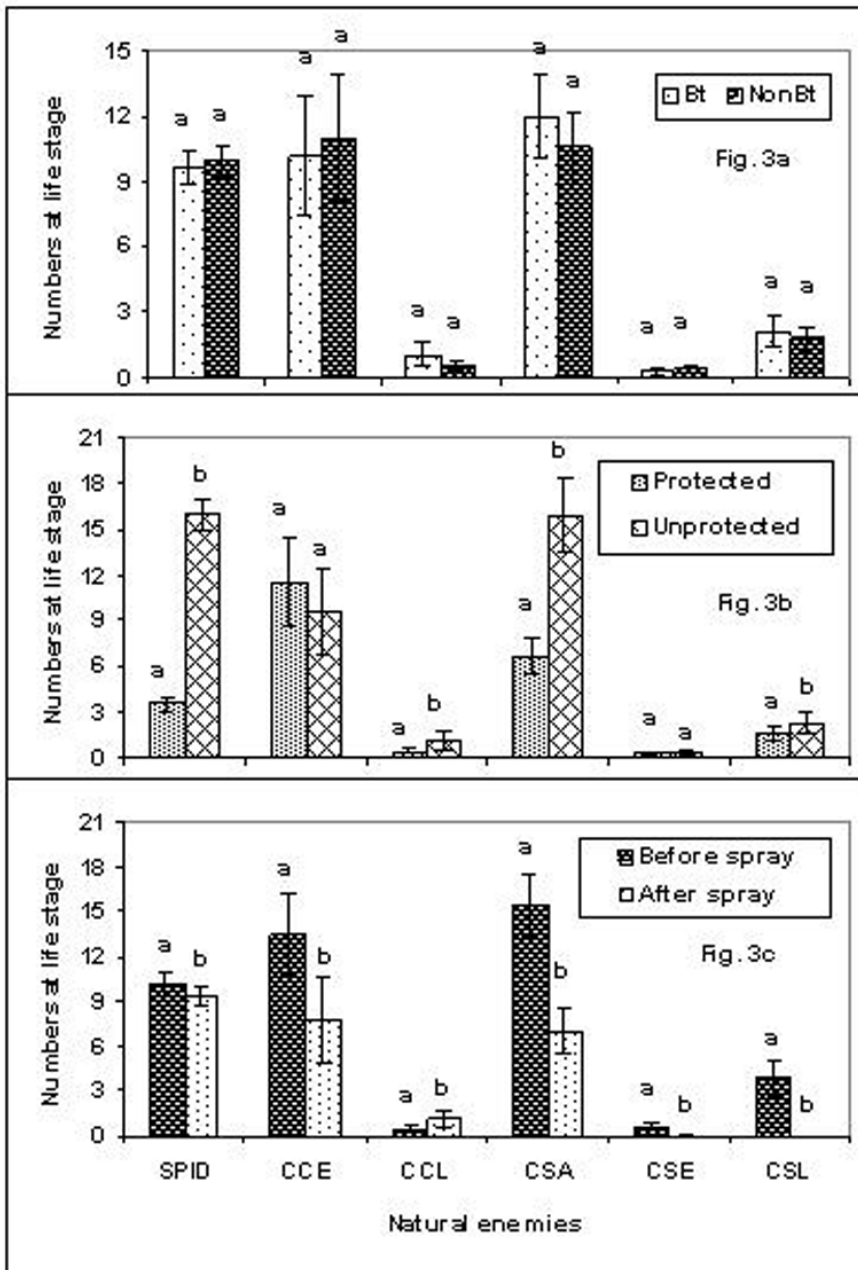


Figure 3. Mean numbers of natural enemies ( $\pm$  SE) in *Bt*-transgenic and non-transgenic cottons (3a), under protected and unprotected (3b), and before and after insecticide sprays (3c). SPID = Spiders plants<sup>-10</sup>. CCE = *Cheilomenes sexmaculatus* eggs plants<sup>-100</sup>. CCL = *C. sexmaculatus* grubs plants<sup>-100</sup>. CSA = *C. sexmaculatus* adults plants<sup>-100</sup>. CSE = *Chrysopa* spp. eggs plants<sup>-100</sup>. CSL = *Chrysopa* spp. grubs plants<sup>-100</sup>. The paired bars following the same letter are nonsignificant at  $P \leq 0.05$ .

### ***Influence of Bt-transgenic cottons on abundance of generalist predators***

There were no significant differences in numbers of coccinellid eggs, grubs and adults; chrysopid eggs and grubs; and the spiders between *Bt*-transgenic and non-transgenic cottons (Figure 3a). No apparent differences have been reported earlier in the abundance of predators in *Bt*-transgenic and non-transgenic cotton under field conditions (Naranjo 2005, Sharma and Pampapathy 2006, Sharma *et al.* 2007, Dhillon and Sharma 2010). Adverse effects of *Bt* toxins on *C. sexmaculatus* on ingestion of *Bt*-fed aphids are unlikely, while direct exposure to *Bt* toxins or predation on *H. armigera* on *Bt*-transgenic plants might have some adverse effects on the activity and abundance of the ladybird, *C. sexmaculatus* (Dhillon and Sharma 2009). The numbers of coccinellid grubs ( $F_{1, 636} = 6.84$ ,  $P = 0.009$ ) and adults ( $F_{1, 636} = 79.70$ ,  $P < 0.001$ ); chrysopid grubs ( $F_{1, 636} = 7.98$ ,  $P = 0.005$ ); and the spiders ( $F_{1, 636} = 1297.58$ ,  $P < 0.001$ ) were significantly greater in unprotected than in insecticide protected plots (Table 1; Figure 3b), indicating significant adverse effects of insecticides on the natural enemies. Numbers of coccinellid eggs ( $F_{1, 636} = 16.05$ ,  $P < 0.001$ ), grubs ( $F_{1, 636} = 51.53$ ,  $P < 0.001$ ), and adults ( $F_{1, 636} = 71.51$ ,  $P < 0.001$ ); chrysopid eggs ( $F_{1, 636} = 8.86$ ,  $P = 0.003$ ) and grubs ( $F_{1, 636} = 5.17$ ,  $P = 0.017$ ); and the spiders ( $F_{1, 636} = 4.51$ ,  $P = 0.034$ ) were significantly greater before insecticide sprays than after insecticide application (Figure 3c). Similar reduction in numbers of generalist predators under insecticide protection has been reported by Sharma *et al.* (2007). Earlier field studies have demonstrated that by midseason the population density of predators such as ladybird beetles, lacewings, spiders, and *Orius similis* Zheng, in *Bt* cotton is significantly higher than in conventional cotton treated with insecticides for the control of *H. armigera* (Wu and Guo 2005). The present studies revealed that the abundance of generalist predators was significantly lower in insecticide protected than in unprotected cottons, suggesting that insecticides have much greater negative effects on the natural enemies than those of *Bt* cotton.

### ***Effect of Bt transgenic cottons on bollworm damage and seedcotton yield***

The *Bt*-transgenic ( $F_{1, 12} = 32.05$ ;  $P < 0.001$ ) and the insecticide protected cottons ( $F_{1, 12} = 61.07$ ;  $P < 0.001$ ) exhibited significantly lower bollworm damage in mature opened bolls than the non-transgenic and the unprotected cottons. The percentage bollworm damage in mature opened bolls was significantly lower in MECH 12 as compared to the other test genotypes (Table 2). Similar reduction in bollworm damage and yield benefits of *Bt*-transgenic cotton have also been reported earlier (Sharma and Pampapathy 2006, Dhillon and Sharma 2010). The bollworm damage in green bolls of *Bt*-transgenic ( $F_{1, 12} = 4.24$ ,  $P = 0.042$ ) and insecticide protected cottons ( $F_{1, 12} = 7.19$ ,  $P = 0.008$ ) was also significantly lower than in the non-*Bt* and unprotected cottons (Table 2). Seedcotton yield ( $F_{3, 12} = 11.49$ ,  $P < 0.001$ ) was significantly higher in RCH 2 and MECH 184 as compared to the other genotypes tested (Table 2). The seedcotton yields of *Bt*-transgenic ( $F_{1, 12} = 25.48$ ,  $P < 0.001$ ) and insecticide protected cottons ( $F_{1, 12} = 174.64$ ,  $P < 0.001$ ) were significantly greater than that of non-*Bt* and unprotected cottons (Table 2), indicating significant contribution of *Bt*-technology in increasing the productivity of cotton. The present studies suggested that *Bt*-transgenic cotton is effective for the management of bollworms and results in a significant increase in seedcotton yield, without any apparent effects on the nontarget insects and natural enemies, and such effects if any, are much lower than those of insecticides.

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Table 2. Boll damage by *Helicoverpa armigera* and seedcotton yields in *Bt*-transgenic and non-transgenic cottons under protected and unprotected conditions

Genotypes	Open damaged bolls (%)				Green damaged bolls (%)				Seedcotton yield (kg/ha)			
	<i>Bt</i> -transgenic		Non-transgenic		<i>Bt</i> -transgenic		Non-transgenic		<i>Bt</i> -transgenic		Non-transgenic	
	P	UP	P	UP	P	UP	P	UP	P	UP	P	UP
MECH 12	12.5 ± 5.8	25.3 ± 7.6	22.4 ± 7.7	42.3 ± 1.9	2.4 ± 1.6	2.2 ± 1.4	2.9 ± 1.2	6.6 ± 1.6	2938 ± 465	1143 ± 595	2976 ± 214	716 ± 522
MECH 162	20.8 ± 5.3	45.9 ± 6.7	24.7 ± 2.8	61.0 ± 6.8	0.7 ± 0.5	2.8 ± 1.4	2.0 ± 1.1	4.2 ± 1.1	4139 ± 225	2010 ± 332	3244 ± 643	1330 ± 171
MECH 184	20.0 ± 2.3	34.6 ± 5.0	38.6 ± 6.1	43.1 ± 3.6	3.8 ± 2.6	3.1 ± 0.8	2.7 ± 1.1	6.5 ± 1.9	3459 ± 369	2745 ± 475	2817 ± 240	2101 ± 602
RCH 2	28.0 ± 7.6	39.5 ± 4.3	41.6 ± 8.4	47.3 ± 4.9	1.5 ± 1.3	3.0 ± 1.8	1.4 ± 1.0	7.0 ± 3.0	4300 ± 477	2409 ± 253	3403 ± 475	1450 ± 356
Mean	20.3 ± 5.3	36.3 ± 5.9	31.8 ± 6.3	48.4 ± 4.3	2.1 ± 1.5	2.8 ± 1.4	2.3 ± 1.1	6.1 ± 1.9	3709 ± 381	2077 ± 414	3110 ± 393	1399 ± 413
LSD (P = 0.05)	Genotype	<i>Bt</i>	Protection	G x <i>Bt</i> x P	Genotype	<i>Bt</i>	Protection	G x <i>Bt</i> x P	Genotype	<i>Bt</i>	Protection	G x <i>Bt</i> x P
for comparing	5.85**	4.13**	4.13**	11.69	2.34	1.66*	1.66**	4.69	354.4**	250.6**	250.6**	708.7

\*, \*\* = Significant at  $P \leq 0.05$ , and  $0.01$ , respectively. NS = Nonsignificant at  $P \leq 0.05$ . G = Genotypes. P = Protected. UP = Unprotected.

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