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

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ABSTRACT

Genetically engineered cottons expressing δ-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 non-target insects, and seedcotton yield under 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 non-target 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, non-target 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, Bollworms, Cotton, Insecticides, Non-target effects, Predators, Sucking insects, Transgenic

Genetically modified plants expressing Bacillus thuringiensis (Bt) δ-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 non-target 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 non-target 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 non-target insect pests and their natural enemies. Therefore, the present studies were undertaken to compare the abundance of target and non-target 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**

Four *Bt*-transgenic cotton hybrids, viz. MECH 12, MECH 162, MECH 184 and RCH 2 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. 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). 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/kg of seed. Six insecticide sprays were applied during the 2005–06 (methomyl 25sp, monocrotophos 36st, methomyl 25sp, cypermethrin 25ec, monocrotophos 36st, and methomyl 25sp), and five sprays each during the 2006–07 (monocrotophos 36st, methomyl 25sp, endosulfan 35ec, cypermethrin 25ec, and methomyl 25sp) and 2007–08 (methomyl 25sp, methomyl 25sp, monocrotophos 36st, 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, respectively.

The abundance of target insect pests [cotton bollworm, *H. armigera*; and spotted bollworm, *Earias vittella* (Fab.)], non-target insect pests [cotton leafhopper, *Amrasca biguttula biguttula* (Ishida); white fly, *Bemisia tabaci* (Gennadius); ash weevils, *Myllocerus* 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.; chrysopid, *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 non-target insects, and the generalist predators before (24 h before spray) and after (48 hr after spray) insecticide sprays.

The numbers of *H. armigera* eggs and larvae were expressed as eggs or larvae/10 plants, 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/10 plants. The cotton aphid infestation was expressed as per cent 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/100 plants. 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/100 plants, while the spiders were expressed as numbers/10 plants.

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.

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® 10th version statistical analysis program (Genstat 2008). The significance of differences between the
<table>
<thead>
<tr>
<th>Target/non-target insects/ natural enemies</th>
<th>Before spray</th>
<th>After spray</th>
<th>LSD (P = 0.05) for comparing</th>
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<tr>
<td><strong>Target insect pests</strong></td>
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<tr>
<td>Bollworm eggs/10 plants</td>
<td>3.9 ± 0.6</td>
<td>2.2 ± 0.4</td>
<td>4.0 ± 0.7</td>
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<td>Bollworm larvae/10 plants</td>
<td>1.1 ± 0.3</td>
<td>0.8 ± 0.2</td>
<td>4.4 ± 0.6</td>
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<td>Spotted bollworm damage (%)</td>
<td>0.01 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.2 ± 0.1</td>
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<td><strong>Non-target insect pests</strong></td>
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<tr>
<td>Cotton leafhoppers/10 plants</td>
<td>36.5 ± 3.1</td>
<td>46.3 ± 3.6</td>
<td>36.5 ± 3.0</td>
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<td>Whiteflies/10 plants</td>
<td>10.2 ± 1.5</td>
<td>4.6 ± 0.6</td>
<td>9.8 ± 1.5</td>
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<td>Aphids infested plants (%)</td>
<td>20.9 ± 3.5</td>
<td>14.7 ± 3.2</td>
<td>21.7 ± 3.6</td>
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<td>Ash weevils/100 plants</td>
<td>16.8 ± 2.1</td>
<td>41.8 ± 5.0</td>
<td>14.9 ± 2.2</td>
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<td>Green bug/100 plants</td>
<td>1.0 ± 0.3</td>
<td>6.7 ± 1.5</td>
<td>0.8 ± 0.3</td>
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<td>Red cotton bug/100 plants</td>
<td>0.1 ± 0.1</td>
<td>13.9 ± 0.8</td>
<td>0.2 ± 0.1</td>
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<td>Dusky cotton bug/100 plants</td>
<td>0.1 ± 0.1</td>
<td>0.5 ± 0.4</td>
<td>0.2 ± 0.2</td>
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<td><strong>Natural enemies</strong></td>
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<tr>
<td>Chrysopid eggs/100 plants</td>
<td>19.2 ± 4.0</td>
<td>9.0 ± 1.8</td>
<td>15.5 ± 3.1</td>
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<td>Chrysopid grubs/100 plants</td>
<td>0.9 ± 0.3</td>
<td>0.2 ± 0.2</td>
<td>0.6 ± 0.2</td>
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<td>Coccinellid adults/100 plants</td>
<td>13.6 ± 2.2</td>
<td>18.9 ± 2.3</td>
<td>11.0 ± 1.8</td>
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<tr>
<td>Coccinellid eggs/100 plants</td>
<td>0.5 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.8 ± 0.3</td>
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<tr>
<td>Coccinellid grubs/100 plants</td>
<td>3.4 ± 1.0</td>
<td>5.1 ± 1.7</td>
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<td>Spiders/10 plants</td>
<td>5.9 ± 0.7</td>
<td>14.0 ± 0.9</td>
<td>6.0 ± 0.7</td>
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*, ** = Significant at P = 0.05, and 0.01, respectively; NS, Nonsignificant at P = 0.05; P, protected; UP, unprotected; Bt, Bt-transgenic; Non-Bt, non-transgenic; Spray, comparison between before and after insecticide sprays.
treatments and their interactions were judged by F-test at \( P = 0.05 \), and the treatment means were compared by least significant difference (LSD) at \( P = 0.05 \).

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 (\( F_{1,636} = 29.72, P < 0.001 \)) (Fig 1b), and their density was greater before insecticide application than after the insecticide sprays (\( F_{1,636} = 31.51, P < 0.001 \)) (Fig 1c). The numbers of *H. armigera* larvae were significantly more in non-Bt than in Bt cottons (\( F_{1,636} = 108.97, P < 0.001 \)) (Fig 1a). The insecticide protected plants had lower numbers of *H. armigera* larvae as compared to unprotected plants (\( F_{1,636} = 8.11, P = 0.004 \)) (Fig 1b). The numbers of *H. armigera* larvae before insecticide application were significantly more than after the insecticide sprays (\( F_{1,636} = 13.96, P < 0.001 \)) (Fig 1c). The spotted bollworm damage was significantly lower in Bt-transgenic cottons (\( F_{1,636} = 8.11, P = 0.004 \)) (Fig 1b). The numbers of *A. gossypii* eggs/10 plants were statistically similar in Bt and non-Bt cottons (\( F_{1,636} = 0.09, P = 0.767 \)) (Fig 2a), and *A. gossypii* infestation was significantly reduced after insecticide application (\( F_{1,636} = 182.85, P < 0.001 \)) (Fig 2c). Aphid infestation was greater in insecticide protected than the unprotected plants (\( F_{1,636} = 21.98, P < 0.001 \)) (Fig 2b), indicating resurgence of cotton aphid due to insecticide application. There were no significant differences in numbers of ash weevils (\( F_{1,552} = 0.84, P = 0.360 \) and red cotton bugs (\( F_{1,552} = 1.74, P = 0.187 \)) between Bt and non-Bt cottons (Fig 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 (Fig 2b, 2c). There were no significant differences in numbers of dusky cotton bugs and green bugs on Bt and non-Bt cottons (Fig 2a), both before and after insecticide sprays (Fig 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 (Fig 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).

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 (Fig 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 Pamapathy 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; Fig 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 (Fig 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 mid-season 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

Fig. 2 Mean numbers of nontarget insect pests (± SE) in Bt-transgenic and non-transgenic cottons (2a), under protected and unprotected (2b), and before and after insecticide sprays (2c). ABB, cotton leafhoppers/10 plants. BT, whiteflies/10 plants. AG, plants with aphid infestation (%). AW, ash weevils/100 plants. NV, green bugs/100 plants. DK, red cotton bugs/100 plants. OL, dusky cotton bugs/100 plants. The paired bars following same letter are non-significant at $P = 0.05$.

Fig. 3 Mean numbers of natural enemies (± SE) in Bt-transgenic and non-transgenic cottons (3a), under protected and unprotected (3b), and before and after insecticide sprays (3c). SPID, Spiders/10 plants; CCE, Cheilomenes sexmaculatus eggs/100 plants; CCL, C. sexmaculatus grubs/100 plants; CSA, C. sexmaculatus adults/100 plants; CSE, Chrysopa spp. eggs/100 plants; CSL, Chrysopa spp. grubs/100 plants. The paired bars following the same letter are nonsignificant at $P = 0.05$. 
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 Pamapathy 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 indicated 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 non-target insects and natural enemies, and such effects if any, are much lower than those of insecticides.

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