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Impact of *Bt*-engineered cotton on target and non-target arthropods, toxin flow through different trophic levels and seedcotton yield

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Abstract: Insect-resistant genetically engineered cottons expressing ?-endotoxins from the bacterium, *Bacillus thuringiensis* (Bt) have been adopted on a large scale worldwide. The effects of *Bt* cotton on non-target insect pests, generalist predators, arthropod diversity and toxin flow through different trophic levels under insecticide protected and unprotected conditions was studied. The populations of major non-target insect pests (leafhoppers, whiteflies, ash weevils, aphids, dusky and red cotton bug, and green bug) and the generalist predators (ladybirds, chrysopids, and spiders) did not differ significantly between the *Bt* and non-*Bt* cottons, while their numbers were lower in insecticide protected than under unprotected conditions, except for aphids and whiteflies. Although, *Bt* toxin was detected in some insect species, no significant differences were observed in their abundance on Bt and non-Bt cottons. Species richness and diversity of plant inhabiting and soil dwelling arthropods was similar in Bt-transgenic and non-transgenic cottons, except in a few cases. The *Bt*-transgenic cotton was effective for the management of bollworms, without any major adverse effects on the non-target arthropods.

Key words: *Bt*-Cotton, non-target effects, arthropod biodiversity, toxin flow, seedcotton yield

Introduction

Genetically modified cotton expressing *Bacillus thuringiensis* (*Bt)* genes with resistance to bollworms have been commercialized for cultivation in several countries, such as in USA (1996), Mexico (1996), Australia (1996), China (1997), Argentina (1998), South Africa (1998), Colombia (2002), India (2002) and Brazil (2005), and occupies 43 per cent (15 m ha of the total area of 35 m ha) of the total global cotton area (James, 2007). India ranks first in the world having 6.2 m ha area under Bt-cotton (66% of total area), followed by China (3.8 m ha) (Manjunath, 2008). Considerable information has been generated on the relative efficacy of transgenic cottons against the target insect pests and their non-target effects in USA, Australia, and China (Pray et al., 2002; Wu *et* al., 2003; Naranjo, 2009), but little information is available on the effect of transgenic cottons on arthropod biodiversity in the tropics, where the transgenic cultivars have been released for cultivation only recently (Qaim and Zilberman, 2003). 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. Therefore, due to multiplicity of crops and cropping systems (mono-, mixed-, inter-, relay-, and sequential-cropping systems), the performance and interactions of transgenic crops in different agro-ecosystems are likely to be quite complex. Therefore, the present studies were undertaken to monitor the *Bt*-toxin flow in the insect fauna through different trophic levels, and compare the abundance of target and non-target arthropods, and seedcotton yield under insecticide protected and unprotected conditions. Such information will be useful to compare relative effects of transgenic cultivars and insecticide sprays in the ecosystem for sustainable crop production.

Material and methods

The *Bt*-transgenic and non-transgenic cotton hybrids were grown under field conditions on deep black soils (Vertisols) at the International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India following normal agronomic practices recommended for raising the crop.

The *Bt*-transgenic and their non-transgenic versions (Mech 12, Mech 162, Mech 184, and RCH 2) were planted in two sets, in a randomized complete block design in three replications. One set was fully protected (seed treatment + need based insecticide application), while another set was completely unprotected. The seeds of each genotype were sown in 4-row plots of 4 m length, on ridges 75 cm apart, with plant-plant spacing of 50 cm. The seeds of the cotton hybrids in protected plots were treated with imidacloprid @ 2 g per kg of seed, followed by six insecticide sprays during the 2005, and five sprays each during the 2006 and 2007 cropping seasons at fortnightly intervals starting from 75 days after seedling emergence (DAE) to 135 DAE, depending on severity of insect damage. The observations were recorded on the abundance of cotton bollworm, nontarget insect pests, and the generalist predators on 5 randomly tagged plants in the middle two rows of each plot at fortnightly intervals, 24 h before and 48 h after insecticide sprays. Leafhopper and whitefly adults and nymphs were recorded on the undersurface of the top five fully expanded leaves, while the rest of the insects were recorded on the whole plant. Numbers of all the insect pests and the generalist predators were expressed as numbers per 100 plants, while the plants infested with aphids were recorded in percentage. The data on total numbers of mature bolls, and those damaged by bollworms were recorded on 5 tagged plants at maturity. Seedcotton was picked-up manually twice from each plot, dried in the sun and weighed, and expressed as kg ha^{-1}. The data were subjected to analysis of variance using GenStat® 10th statistical analysis program. Significance of differences between treatments were judged by F-test, and the means were compared by LSD at P = 0.05.

The Bt-transgenic and non-transgenic version of Mech 12 were planted in two modules: i) protected (seed treatment + insecticide sprays starting from 60 DAE), and ii) unprotected, each on a plot size of 325 m^2. Both Bt and non-Bt cotton plots were divided in five subplots of 4 rows, 4 m long by leaving a 4 m boundary all around the plot for sampling arthropods. The abundance of arthropod species were recorded at five fortnightly intervals starting from 75 to 135 DAE on five Bt and the non-Bt cotton plants tagged at random.

The Bt-transgenic MRC 7201 BGII and the non-transgenic counterpart were sown in two modules: i) protected (seed treatment + insecticide sprays starting from 60 DAE, and ii) unprotected, each on a plot size of 325 m^2. A total of five wet pitfall traps were installed (equidistant from each other) in each plot to collect the soil dwelling arthropods. The traps were operated at fortnightly intervals, and the protected plots were insecticide sprayed (48 h before the trap installation) throughout the crop season starting from 30 to 165 DAE. The arthropod species collected from the traps were recorded and sorted according to insect orders.

Diversity index of plant inhabiting and the soil dwelling insects was computed separately using the Simpson (1951) formula:

Simpson's index of diversity = 1 - Simpson's index [D = S n (n-1) / S N (N-1)], where, n = Total number of insects of a particular species, and N = Total number of insects of all species

The insect species settled/visiting the *Bt* Mech 12 and non-Bt Mech 12 cotton hybrids were collected in 50 ml plexi glass vials, and stored at -20°C. About 50 mg of each insect species (numbers varied according to the insect size), were weighed in Eppendorf tubes and crushed (whole body) in PBS buffer in a ratio of 1: 10 (insect sample: buffer) with a plastic pastel to detect the *Bt* protein in the insect body. The semi-quantitative ELISA (Agdia®) was performed using the procedure given by Sharma et al. (2008), along with negative and positive controls, and 0.5, 2.5 and 5.0 ppb Bt standards.

Results and discussion

The numbers of H. *armigera* larvae were significantly more in non-*Bt* than in *Bt* cottons, however, no significant differences were recorded in egg laying by the adult females between *Bt* and non-*Bt* cottons (Table 1). The numbers of eggs laid by H. *armigera* were significantly more, while larvae were lower in insecticide protected plots as compared to that on the unprotected plots. However, the numbers of H. armigera eggs and larvae were significantly greater before insecticide application than after the insecticide sprays. More egg laying by H. *armigera* on insecticide protected plants might be because of better crop growth as a result of reduced damage by other insect pests. Similar results were also obtained in earlier studies by Sharma and Pampapathy (2006) and Dhillon and Sharma (2009b). The bollworm damage and seedcotton yield in Bt-transgenic (Fig. 1a, 1b) and the insecticide protected (Fig. 1c, 1d) cottons was significantly lower than that in non-transgenic unprotected cottons. Earlier studies have also suggested that the deployment of *Bt*-cotton in combination with insecticides is effective for bollworm control, and produces more seedcotton

Table 1. Populations of Helicoverpa armigera, nontarget insect pests, and the generalist predators per hundred plants of Bt and non-Bt cottons under insecticide protected and unprotected conditions (2005-07 rainy seasons)

Test insects	Before spray				After spray				LSD (P = 0.05) for comparing			
	Bt + P	Bt + UP	NBt + P	NBt + UP	Bt + P	Bt + UP	NBt + P	NBt + UP	Bt	Protect	Spray	Bt x P x S
Target insect pest												
H. armigera eggs	39 ± 6	22 ± 4	40 ± 7	26 ± 4	22 ± 5	13 ± 2	25 ± 6	15 ± 4	NS	4.5**	4.5**	NS
H. armigera larvae	11 ± 3	8 ± 2	44 ± 6	24 ± 4	6 ± 2	10 ± 3	23 ± 6	24 ± 4	3.6**	3.6**	3.6**	NS
Nontarget insect pests												
Leafhoppers	365 ± 31	463 ± 36	365 ± 30	484 ± 37	134 ± 18	582 ± 51	131 ± 19	592 ± 49	NS	28.4**	28.4**	NS
Whiteflies	102 ± 15	46 ± 6	98 ± 15	48 ± 6	60 ± 10	48 ± 8	62 ± 10	51 ± 7	NS	9.8**	9.8**	NS
Aphid infested plants	21 ± 4	15 ± 3	22 ± 4	16 ± 3	6 ± 3	1 ± 0.3	6 ± 3	0.5 ± 0.3	NS	2.4**	2.4**	NS
Ash weevils	17 ± 2	42 ± 5	15 ± 2	37 ± 5	1 ± 0.3	31 ± 5	0.2 ± 0.1	32 ± 5	NS	3.5**	3.5**	NS
Green bug	1 ± 0.3	7 ± 2	1 ± 0.3	8 ± 2	0 ± 0	11 ± 3	0 ± 0	8 ± 2	NS	1.3**	1.3**	NS
Red cotton bug	0.1 ± 0.1	14 ± 1	0.2 ± 0.1	22 ± 12	0 ± 0	10 ± 5	0 ± 0	10 ± 5	NS	4.8**	4.8**	NS
Dusky cotton bug	0.1 ± 0.1	0.5 ± 0.4	0.2 ± 0.2	2 ± 1	0 ± 0	25 ± 10	0 ± 0	34 ± 16	NS	6.8**	6.8**	NS
Generalist predators												
Chrysopid eggs	19 ± 4	9 ± 2	16 ± 3	10 ± 2	4 ± 1	8 ± 4	7 ± 3	11 ± 4	NS	NS	3.00**	NS
Chrysopid larvae	1 ± 0.3	0.2 ± 0.2	1 ± 0.2	0.3 ± 0.1	0.2 ± 0.2	3 ± 1.4	0.1 ± 0.1	2 ± 1	NS	0.40**	0.40*	NS
Coccinellid adults	14 ± 2	19 ± 2	11 ± 2	18 ± 2	1 ± 0.4	14 ± 3	1 ± 0.5	12 ± 2	NS	2.00**	2.00**	NS
Coccinellid eggs	1 ± 0.2	0.4 ± 0.2	1 ± 0.3	1 ± 0.4	0 ± 0	0.2 ± 0.2	0 ± 0	0 ± 0	NS	NS	0.30**	NS
Coccinellid larvae	3 ± 1	5 ± 2	3 ± 1	4 ± 1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	NS	1.00**	1.00**	NS
Spiders	59 ± 7	140 ± 9	60 ± 7	147 ± 9	12 ± 3	174 ± 11	12 ± 3	178 ± 10	NS	6.8**	6.8*	NS

*, ** = Significant at P = 0.05, and 0.01, respectively, NS = Non-significant at P = 0.05, P = Protected, UP = Unprotected, Bt = Bt-transgenic, NBt = Non-transgenic, Spray = Comparison between before and after insecticide sprays

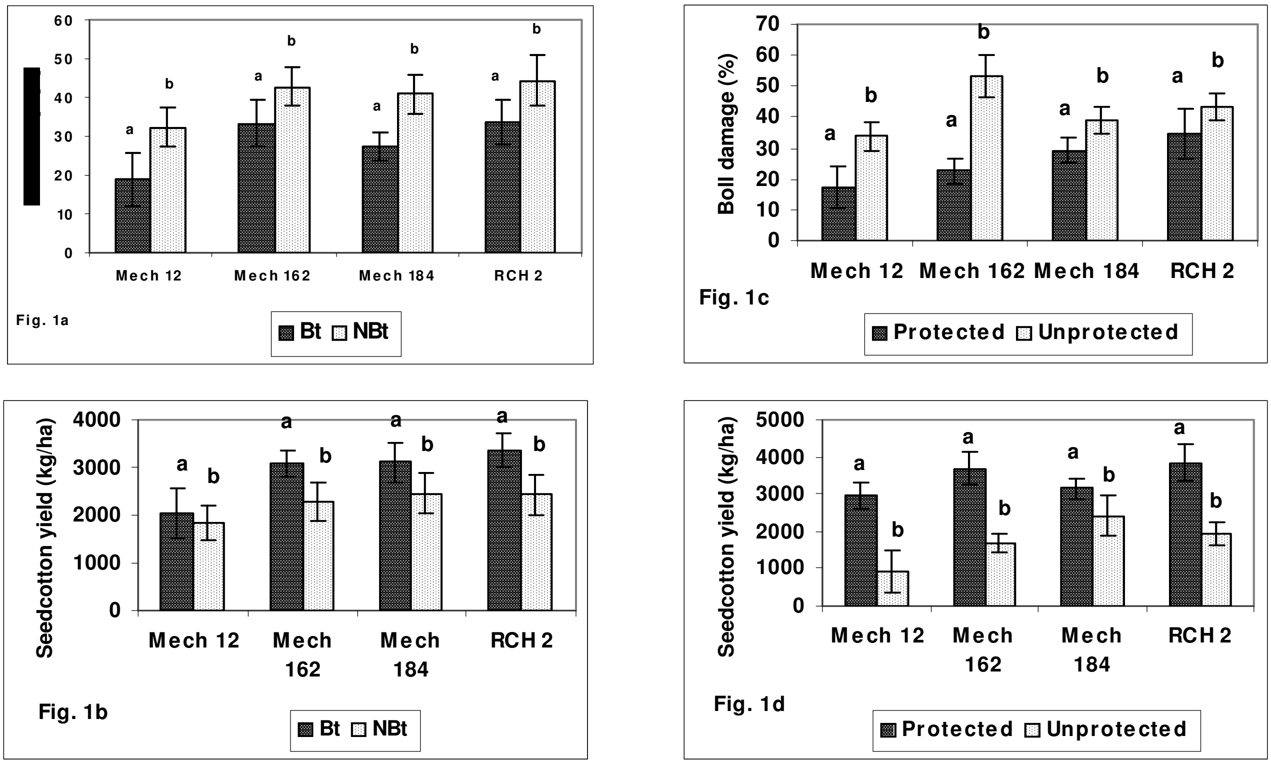

Fig. 1. Boll damage by bollworms **(1a)** and seedcotton yield **(1b)** in *Bt*-transgenic and non-transgenic cottons under insecticide protected and unprotected conditions **(1c, 1d)**. The paired bars with different letters are significantly different at $P < 0.05$.

at lower rates of insecticide application (Pray *et al.,* 2002; Sharma and Pampapathy, 2006).

There were no significant differences in numbers of leafhoppers, whiteflies, aphid infestation, ash weevils, green bugs, red and dusky cotton bugs between *Bt* and non-*Bt* cottons (Table 1). However, the numbers of these insect pests were significantly greater in unprotected than on the protected plots, except for whiteflies and aphids. The insect counts before and after insecticide sprays, further confirmed that the insecticide application significantly lowered the numbers of all the insect pests under observation. There was no evidence of increased susceptibility of Bt-cottons to nontarget insects such as leafhoppers, red cotton bugs, dusky cotton bugs, green bugs, and ash weevils, however, resurgence of some insect species in insecticide protected plots may be due to reduced numbers of predators as was observed in case of whiteflies and aphids. Earlier large-scale studies have also confirmed the negative effects of broad-spectrum insecticides on insect communities in both Bt and non-Bt crops (Whitehouse *et al.,* 2005; Dhillon and Sharma, 2009b; Naranjo, 2009).

Concerns have been expressed regarding the possible effects of Bt-transgenic crops on the nontarget natural enemies through decrease in density of immature stages of insects that serve as a food for parasitic and predatory arthropods (Romeis et al., 2006; Sharma et al., 2007, 2008; Dhillon and Sharma, 2009a). Adverse effects of Bt toxins on the ladybirds on ingestion of Bt-fed aphids are unlikely, but predation on young bollworm larvae consuming -cotton might cause some adverse effects on the ladybirds (Dhillon and Sharma, 2009a). However, some of the parasitoids might survive on the alternate host insects under diverse crops and cropping systems (Dhillon and Sharma, 2007). No significant differences were observed in the numbers of coccinellids, chrysopids and the spiders between Bt and non-Bt cottons, however, their numbers were significantly lower in insecticide protected and after insecticide application than in unprotected and before insecticide sprays (Table 1). Earlier field trials have also demonstrated that by mid-season, the population densities of generalist predators in Bt-cotton are significantly higher than in conventional cottons treated with insecticides for control of H. armigera (Pray *et al,* 2002; Sharma *et al.,* 2007).

Species richness of plant inhabiting and of soil dwelling arthropods was similar in Bt and non-Bt cotton plots. A total of 18 arthropod species of plant inhabiting, and 64 species of soil dwelling arthropods were observed and their relative abundance was recorded during the experimental period. Simpsons's index of diversity for hemipterans in Bt-cotton under unprotected conditions was lower than in Bt-cotton under protected, and non-Bt cotton under insecticide protected and unprotected conditions, which was largely due to high numbers of leafhoppers in the Bt-cotton under unprotected conditions (Fig. 2). Similarly, the diversity index of coleopterans in non-Bt cotton under insecticide protected conditions was lower than in unprotected Bt and non-Bt cottons. This may be due to more numbers of coleopterans in non-Bt cotton under insecticide protected conditions. However, no significant influence of Bt-cotton and/or insecticide application was observed on the

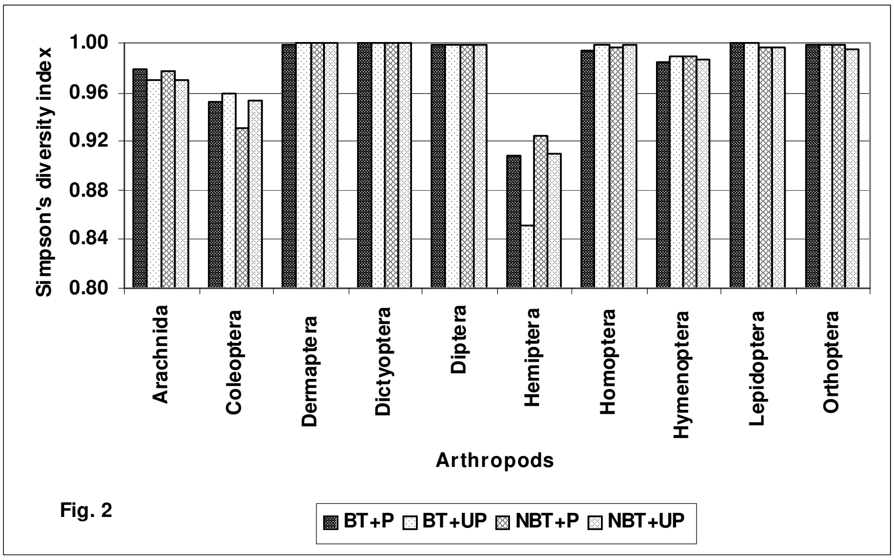

Fig. 2. Simpson's diversity index of plant inhabiting and soil dwelling arthropods in *Bt* and non-*Bt* cottons under insecticide protected and unprotected conditions.

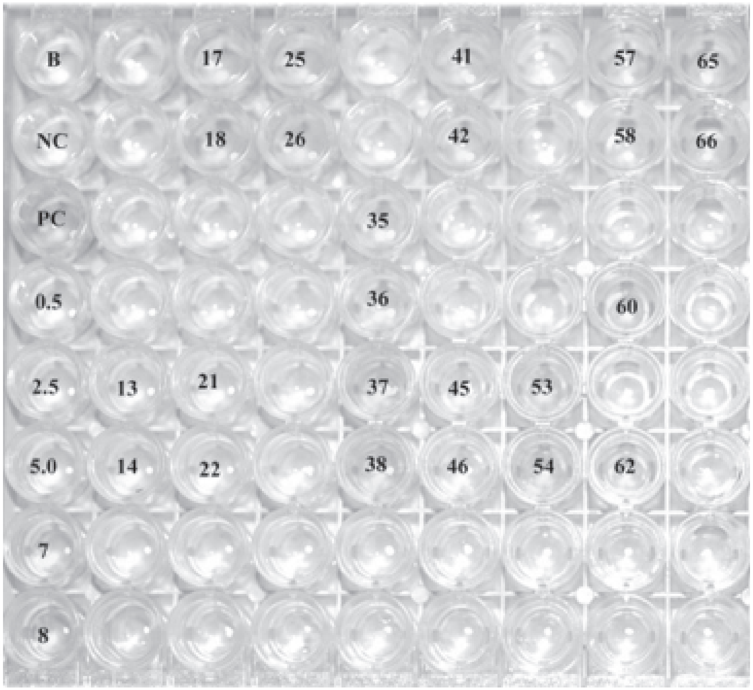

Fig. 3. *Bt*-toxin flow from transgenic cotton in insect fauna. Insect species imbibed *Bt* toxin from transgenic cotton genotype Mech 12: 7, 8: Bt cotton leaf, 13, 14: Spiders, 17, 18: Surface grasshopper, 21, 22: Helicoverpa larvae, 25, 26: Green Grasshopper, 35, 36: Dusky cotton bug (non-Bt), 37, 38: Dusky cotton bug (Bt), 41, 42: Ash weevil, 45, 46: Coccinellid adult, 53, 54: Cotton leafhopper, 57, 58: Thrips, 60: Chrysopid larvae, 62. Coccinellid larvae, 65, 66: Grasshopper.

diversity index of other plant inhabiting and soil dwelling arthropods. Earlier studies have reported higher number of arthropods in *Bt*-cotton fields under reduced or no insecticide application than in the conventional insecticide protected cotton (Pray et al., 2002; Sisterson et al., 2004; Naranjo, 2009). A total of 30 insect species each from *Bt* and non-*Bt* cottons were tested for the presence of *Bt*-toxin using qualitative ELISA (Fig. 3). Amongst these, spiders, grasshopper and katydid species, blister beetles, red and dusky cotton bugs, ash weevils, cotton leafhoppers, thrips, and chrysopid larvae had >5.0 ppb Bt toxin, while H. armigera larvae and coccinellid adults and larvae had 2.5 to 5.0 ppb Bt-toxin. However, no Bt-toxin was detected in insects colleted from the non-Bt cotton, and some bug and grasshopper species, damsel and dragon flies, and aphids collected from Bt-cotton.

The *Bt*-transgenic cotton plays a significant role in reducing the bollworm damage, dosage and frequency of insecticide application, and result in increased seedcotton yield, without any apparent effects on the non-target insect pests and the generalist predators. Although, Bt toxin was detected in some insect species, no significant differences were observed in their abundance on Bt and non-Bt cottons. The species richness and the diversity index of the plant inhabiting and soil dwelling arthropod species was similar in Bt and non-Bt cottons, except in a few cases. The breadth of coverage of biodiversity in general and arthropods in particular for biosafety studies of Bt-transgenic crops is not sufficient under tropics due to diverse cropping systems and agro-ecosystems, therefore, there is a continuing need to monitor the populations of non-target insect species for a longer period of time to understand the long-term impact of Bt crops on insect biodiversity and population dynamics for sustainable crop production.

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